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This project examined a new agent that combines a transition metal anticancer agent CDDP with an antiestrogen (tamoxifen analogs) in the breast and ovarian cancer cell lines. The tamoxifen portion of the conjugate would serve as a carrier to selectively accumulate CDDP portion in the nucleus of ER-positive cancer cells, while CDDP alone does not show any selectivity between normal and cancer cells. The combined agent would result in selective accumulation in estrogen receptor (ER) positive cancer cells and also produce a cytotoxic effect by the reaction of CDDP with DNA. Synergistic inhibition of human cancer cell growth by CDDP and tamoxifen on an intermolecular basis has been demonstrated in vivo. The uniqueness of the present proposal is the covalent linkage between tamoxifen and platinum, which could be cleaved either enzymatically or chemically. A number of conjugates have been synthesized and characterized. All conjugates show cytotoxicity. A conjugate with greater potency than carboplatin has been observed. However, the potency does not correlate with estrogen receptor binding affinity well. No synergistic effect among the fragments of conjugates has been observed.

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INTRODUCTION

The purpose of this project is to examine a new kind of agents that combine a transition metal anticancer agent CDDP with an antiestrogen (tamoxifen analogs, typical examples shown in **Scheme 1**) in the breast and ovarian cancer cell lines. The tamoxifen portion of the conjugate will serve as a carrier to selectively accumulate CDDP portion in the nucleus of estrogen receptor (ER)-positive cancer cells, while CDDP alone does not show any selectivity between normal and cancer cells. The conjugates would result in selective accumulation in ER-positive cancer cells and also produce a cytotoxic effect by the reaction of CDDP with DNA (**Scheme 3**). Synergistic inhibition of human cancer cell growth by CDDP and tamoxifen on an intermolecular basis has been demonstrated *in vivo*. The uniqueness of the present project is the covalent linkage between tamoxifen and platinum, which could be cleaved either enzymatically or chemically after the conjugates enter the cancer cells. Other examples of conjugates are shown in **Scheme 2**. We have designed and evaluated a number of platinum conjugates in breast and ovarian cancer cell lines.[1] We examined the following two aspects in the research: selective accumulation of CDDP in the nucleus of cancer cells; synergism between CDDP and tamoxifen.

BODY

(1) Synthesis of the Conjugates between Platinum Compounds and Tamoxifen analogs

Results and discussion

A general structure for the compounds synthesized is shown in **Scheme 4**. A retro-synthesis of the targets is shown in **Scheme 5**. **Schemes 6 – 11 show** the actual synthetic steps used to make the target molecules. Each compound has been characterized by ^{1}H, $^{13}C\{^{1}H\}$, elemental analysis, MS analysis, and possibly ^{195}Pt NMR if available. The conjugates are composed of three main functional units, a homing unit, a linker unit, and a warhead unit. The six-carbon linker provides proper length to attach functional groups on both sides through proper protection. The incorporation of the platinum fragment follows the synthesis of carboplatin.[2]

The starting compounds **7a — 7c** have been made by a modified procedure.[3] The reaction used, as illustrated in **Scheme 6**. The Grignard reagents were made by the standard procedure through the bromides with Mg in distilled anhydrous diethyl ether, initiated by I2, in dinitrogen atmosphere. The Grignard reaction normally takes overnight at room temperature. The resultant tertiary aromatic alcohols **8a — 8e** are easily dehydrated and de-protected under mild acidic conditions at elevated temperature, to afford **9a — 9e (Scheme 7)**. Formation of the bromides was accomplished through the reagents of either Ph_3P/CBr_4 or PBr_3, depending on the functional groups at the aromatic systems, to generate **10a — 10e**. The methyl group of the aromatic ethers, was removed by BBr_3 in anhydrous CH_2Cl_2, to give **11a — 11d (Scheme 8)**. The malonate group was introduced through S_N2 reaction with NaH as the base, at room temperature, to generate **12a — 12d**. For selected examples, the aromatic phenol group was alkylated to give **13a**, a close analog of tamoxifen (**Scheme 9**). The esters, **12a — 12d**, were hydrolyzed by KOH in ethanol at reflux. The corresponding free acids were generated by acidification of HCl and extraction in organic solvents, to afford **14a — 14d**. Introduction of platinum fragments into the diacid functional group generated **15a — 15d (Scheme 10)**. However, the same procedure cannot be used to synthesize **17a**. Instead, an alternative procedure

was designed. After hydrolysis of the diester by NaOH, the corresponding sodium salt was transformed into silver salt, which precipitate from aqueous solution. Reaction of the silver salt with appropriate platinum precursor generated the target conjugate **17a** (**Scheme 11**).

Experimental.

(a) Materials. Anhydrous reactions were performed under an inert atmosphere. Unless otherwise noted, starting material, reactant, and solvents were obtained commercially and were used as such and dried by standard means. Organic solutions were dried over magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4), and evaporated on a Buchi tabletop rotary evaporator and under reduced pressure. All reactions were monitored by thin-layer chromatography (TLC). The plates were visualized under UV light (254 nm), or by staining with iodine (I_2). Commercial TLC plates were from Merck. Flash column chromatography was performed on Merck grade 60 silica gel, 230-400 meshes. Mass spectral assays (MS, *m/e*) were obtained using a Finnigan LCQ instrument at the Research Resource Center (RRC) East of the University of Illinois at Chicago. High-resolution mass spectra (HRMS) were obtained either from the RRCE facility or from the Mass facility of the University of Minnesota, twin cities. Nuclear magnetic resonance (NMR) spectra and were obtained on either a Varian VX300 (300 MHz) or a Bruker AM400 (400 MHz) spectrometer. Chemical shifts were measured relative to the internal standards: $CDCl_3$ ($\delta = 7.27$ ppm), MeOD-d_4 ($\delta = 3.31$ ppm), DMSO-d_6 ($\delta = 2.50$ ppm).

(b) Synthetic procedure. 1-Tetrahydropyranyloxy-6-bromohexane (**4**). A solution of 6-bromo-1-hexanol (2.5 g, 13.8 mmol), 3,4-dihydro-2*H*-pyran (1.3 g, 15.3 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 5 mg, 0.02 mmol) in dichloromethane (CH_2Cl_2, 50 ml) was stirred for 5 h under nitrogen. Afterwards, sodium bicarbonate ($NaHCO_3$, 500 mg) and $MgSO_4$ (5.0 g) were added to the reaction mixture and stirred for 20 min. before being filtered on a short pad of Celite/silica gel (1 cm/4 cm) using CH_2Cl_2 as eluent. The filtrate was evaporated to viscous oil, 3.43 g (12.9 mmol, 93.5 % yield), which was used without further purification in the next step. ^{1}H NMR in $CDCl_3$, δ, 4.58 (bt, 1H, -O-**CH**R-O-), 3.86, 3.51 (m, 2H, α-**CH₂** of pyran), 3.75, 3.406 (m, 2H, -**CH₂**O-CHR-O-), 3.42 (t, $^3J_{HH}$ = 6.8 Hz, 2H, -**CH₂**Br), 1.83 – 1.92 (m, 4H, -**CH₂**CH₂Br and -**CH₂** of THP), 1.71 – 1.75 (m, 2H, -**CH₂**CH₂O-CHR-O-), 1.38 – 1.66 (m, 8H, 4 × –**CH₂**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 99.1, 67.6, 62.6, 34.1, 33.0, 31.0, 29.8, 28.2, 25.7, 19.9. Mass: $C_{11}H_{21}O_2Br_1$, Average FW = 265.19, HRMS (TOF MS ES$^+$); $C_{11}H_{21}O_2Br_1Na_1$ $[M + Na]^+$, Calc. Mass 287.0623, Found 287.0630, $C_{11}H_{21}O_2{}^{81}Br_1Na_1$ $[M + Na]^+$, Calc. Mass 289.0602, Found 289.0606.

1-Tetrahydropyranyloxy-6-iodohexane (5). To a solution of crude bromide **4** (3.43 g, 12.9 mmol) in dry acetone was added sodium iodide (2.92 g, 19.5 mmol). The reaction mixture was stirred at 25°C for 5 h. Then most of the solvent was evaporated and the residue was transferred to a 125 mL separatory funnel with ether (50 mL) and water (50 mL). The organic phase was washed with water (3 × 50 mL), dried with $MgSO_4$, filtered, and concentrated to a viscous liquid. The crude iodide **5** (3.26 g, 10.5 mmol, 81.4 % yield) was used as such at the following alkylation step. ^{1}H NMR in $CDCl_3$: δ 4.58 (bt, 1H, -O-**CH**R-O-), 3.87, 3.51 (m, 2H, α-**CH₂** of THP), 3.75, 3.39 (m, 2H, -**CH₂**O-CHR-O-), 3.20 (t, $^3J_{HH}$ = 7.0 Hz, 2H, -**CH₂**I), 1.83 – 1.86 (m, 4H, -**CH₂**CH₂I

and δ-CH_2 of THP), 1.70 – 1.75 (m, 2H, -CH_2CH_2O-CHR-O-), 1.38 – 1.66 (m, 8H, 4 × -CH_2-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 99.1, 67.6, 62.6, 33.7, 31.0, 30.5, 29.7, 25.7, 25.5, 19.9, 7.37.

1,2-Bis(phenyl)-8-tetrahydropyranyloxy-octanone, (7a). To a stirred suspension of sodium hydride (502 mg, 12.6 mmol, 60 % dispersion in mineral oil) in dry tetrahydrofuran (THF, 100 mL) was added rapidly ketone deoxybenzoin (2.0 g, 10.2 mmol). During the addition, bubbles were generated and the color of the reaction mixture turned to light green. After fizzing stopped, the reaction mixture was heated (60°C) on a heating mantle connected to a variable autotransformer for 1.5 hr under a nitrogen atmosphere. After cooling, 1-tetrahydropyranyloxy-6-iodohexane (11.9 mmol) in dry THF was added dropwise and the resulting mixture stirred overnight (16 h) at room temperature (22 °C). At the end of the reaction, the color of the mixture turned to light brown. The reaction progress was checked and confirmed by TLC and ^{1}H NMR. Most of the solvent was then evaporated with a Buchi tabletop rotary evaporator. The dark orange residue was diluted with ether (100 mL) and treated with water (50 mL). The ethereal phase turned yellow and the remaining white powder dissolved into the water phase. Product was extracted into the ethereal phase by washing the mixture with water (4 × 50mL). The ethereal phase was dried with $MgSO_4$ for 1.5 hrs, and evaporated to give orange oil (3.8 g). The product was purified by a flash column chromatography using a silica bed. (hexane:acetone, 95:5). The product was eluted from the fraction number 7 to 20 (15 mL/fraction). The product pool was collected and solvent evaporated. A transparent oily residue obtained (3.7 g, 9.74 mmol). Yield was 95.5 %. Rf is 0.59 with hexane:ethylacetate (8:2). ^{1}H NMR in $CDCl_3$, δ, 7.96 (d, $^3J_{HH}$ = 3.7 Hz, 2H, o-**H**-Ar(A)), 7.48 (t, $^3J_{HH}$ = 3.7 Hz, 1H, p-**H**-Ar(A)), 7.38 (t, $^3J_{HH}$ = 3.8 Hz, 2H, m-**H**-Ar(B)), 7.26 – 7.32 (m, 4H, o, m-**H**-Ar(B)), 7.21 (d, $^3J_{HH}$ = 3.3 Hz, 1H, p-**H**-Ar(B)), 4.51 – 4.55 (m, 2H, -COC**H**R- and –O-C**H**R-O-), 3.84, 3.48 (bt, 2H, α-CH_2 of THP), 3.70, 3.34 (bm, 2H, -CH_2-O-CHR-O-), 2.10 – 2.20 (b, 2H, -COCHR-CH_2-), 1.80 – 1.85 (b, 2H, δ-CH_2 of THP), 1.67 – 1.70 (b, 2H, -CH_2CH_2O-CH-O-), 1.48 – 1.58 (b, 4H, β,γ-CH_2 of THP), 1.24 – 1.33 (b, 6H, 3 × –CH_2-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 200.0, 136.7, 136.9, 132.7, 128.8, 128.5, 128.4, 128.1, 126.8, 98.7, 67.5, 62.3, 53.6, 33.9, 30.7, 29.6, 29.4, 27.6, 26.0, 25.4, 19.6. Mass: $C_{25}H_{32}O_3$, Average FW = 380.53, LRMS; 403.3 $[M + Na]^+$ (100), HRMS; $C_{25}H_{32}O_3Na_1$ $[M + Na]^+$, Calc. Mass 403.2249, Found 403.2242.

1,2-Bis(4-methoxyphenyl)-8-tetrahydropyranyloxy-octanone (7c). To a stirred suspension of sodium hydride (520 mg, 13.0 mmol, 60 % dispersion in mineral oil) in dry tetrahydrofuran (THF, 100 mL) was added rapidly ketone desoxyanizoin (3.1 g, 12.2 mmol). During the addition, bubbles were generated and the color of the reaction mixture turned to light green. After fizzing stopped, the reaction mixture was heated (60 °C) on a heating mantle connected to a variac (variable autotransformer) for 1.5 hr under a nitrogen atmosphere. After cooling, 1-tetrahydropyranyloxy-6-iodohexane (3.8 g, 12.2 mmol) in dry THF was added dropwise and the resulting mixture stirred overnight (16 h) at room temperature (22 °C). At the end of the reaction, the color of the mixture turned to light brown. The reaction progress was checked and confirmed by TLC and ^{1}H NMR. Most of the solvent was then evaporated by a Buchi tabletop rotary evaporator. The dark brown residue was diluted with ether (70 mL) and treated with water (50 mL). The ethereal phase turned greenish yellow and the remaining white powder dissolved into the water phase. Product was extracted into the ethereal phase by washing the mixture with

water (4 × 50mL). The ethereal phase was dried with $MgSO_4$ for 1.5 hrs, and evaporated to give orange oil (7.5 g). The product was purified by a flash column chromatography using a silica bed. (hexane:acetone, 95:5). The product was eluted from the fraction number 7 to 20 (15 mL/fraction). The product pool was collected and solvent evaporated. A transparent oily residue obtained (5.3 g, 12.0 mmol). Yield was 98.4 %. Rf is 0.34 with hexane:ethylacetate (8:2). ^{1}H NMR in $CDCl_3$, δ, 7.95 (d, $^3J_{HH}$ = 4.5 Hz, 2H, o-**H**-Ar(A)), 7.21 (d, $^3J_{HH}$ = 4.3 Hz 4H, o-**H**-Ar(B)), 6.86 (d, $^3J_{HH}$ = 3.6 Hz, 2H, m-**H**-Ar(A)), 6.81 (d, $^3J_{HH}$ = 3.4 Hz, 2H, m-**H**-Ar(B)), 4.55 (bt, $^3J_{HH}$ = 3.4 Hz, 1H, –O-C**H**R-O-), 4.44 (t, $^3J_{HH}$ = 7.3 Hz 1H, -COC**H**R-), 3.86, 3.48 (b, 2H, α-$\mathbf{CH_2}$ of THP), 3.81 (s, 3H, -O$\mathbf{CH_3}$(A)), 3.74 (s, 3H, -O$\mathbf{CH_3}$(B)), 3.69, 3.34 (bm, 2H, -$\mathbf{CH_2}$-O-CHR-O-), 2.11 – 2.13 (b, 2H, -COCHR-$\mathbf{CH_2}$-), 1.77 – 1.85 (b, 2H, δ-$\mathbf{CH_2}$ of THP), 1.67 – 1.73 (b, 2H, -$\mathbf{CH_2}$$CH_2$O-CH-O-), 1.49 – 1.57 (b, 4H, β,γ-$\mathbf{CH_2}$ of THP), 1.23 – 1.34 (b, 6H, 3 × –$\mathbf{CH_2}$-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 199.0, 163.3, 158.6, 132.0, 131.1, 130.1, 129.3, 114.4, 113.8, 99.0, 67.8, 62.5, 55.6, 55.3, 52.5, 34.2, 30.9, 29.8, 29.7, 27.8, 26.3, 25.7, 19.9. Mass: $C_{27}H_{36}O_5$, Average FW = 440.58, LRMS; 463.3 $[M + Na]^+$ (83), 902.9 $[2M + Na]^+$ (100), HRMS; $C_{27}H_{36}O_5Na_1$ $[M + Na]^+$ (100), Calc. Mass 463.2460, Found 463.2462.

4-(2'-Dimethylaminoethoxy)phenylbromide. 4-Bromophenol (5.12 g, 29.6 mmol) was weighed into a 250 mL round bottom flask and dissolved with 100 mL N,N'-dimethylformamide (DMF) in the presence of 10 equivalent powder potassium carbonate (K_2CO_3, 40.9 g, 296 mmol). The solution was heated for 1 h to generate the phenolate. Solution color turned to pink. Add 2-(dimethylamino)ethyl chloride hydrochloride, ($(CH_3)_2NCH_2CH_2Cl\cdot HCl$, 2 eq., 8.1 g, 59.2 mmol) in 70 mL DMF. Color then changed to orange. Reflux the reaction mixture overnight (16 h). After the reaction is completed, filter the solid remnants, evaporate DMF. Add 50 mL of 10 % NaOH to neutralize the product. Extract the product with diethylether (3 × 30 mL). Dry the ethereal phase with $MgSO_4$ for 5 hrs and evaporate ether to get the product (4.81 g, 19.7 mmol, 66.6 % yield). ^{1}H NMR in $CDCl_3$, δ, 7.370 (d, $^3J_{HH}$ = 8.8 Hz, 2H, o-Ar-**H**), 6.82 (d, $^3J_{HH}$ = 8.8 Hz, 2H, m-Ar-**H**), 4.03 (t, $^3J_{HH}$ = 5.8 Hz, 2H, -O$\mathbf{CH_2}$$CH_2$N), 2.72 (t, $^3J_{HH}$ = 5.7 Hz, 2H, -O$CH_2$$\mathbf{CH_2}$N), 2.34 (s, 6H, -N$(\mathbf{CH_3})_2$). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 158.2, 132.4, 132.4, 116.6, 66.4, 58.4, 46.1. Mass(*m/e*), $C_{10}H_{14}O_1N_1Br_1$, Average FW = 244.13, LRMS; $[M + H]^+$ 244.1(100), 246.1(77). HRMS; $C_{10}H_{15}N_1O_1Br_1$ $[M + H]^+$ (100), Calc. Mass 244.0337, Found 244.0336, 244.0337 (100), 246.0322 (94).

4-(2'-Dimethylaminoethoxy)phenylmagnesiumbromide. Typically, N,N'-dimethylaminoethanebromobenzene (2.5 g, 10.3 mmol) was mixed with Mg turnings (340 mg, 14 mmol) in 50 mL of dry tetrahydrofuran (THF) in a glove box. A small amount of I_2 was added to initiate the radical reaction. The Grignard reagent was ready after stirring the reaction mixture overnight (16 h). ^{1}H NMR in $CDCl_3$, δ, 7.32 (d, $^3J_{HH}$ = 8.8 Hz, 2H, m-Ar-**H**), 7.03 (t, 1H, p-Ar-**H**), 6.93 (d, $^3J_{HH}$ = 8.4 Hz, 2H, o-Ar-**H**), 4.49 (t, $^3J_{HH}$ = 4.6 Hz, 2H, -O$\mathbf{CH_2}$$CH_2$N), 3.40 (bt, $^3J_{HH}$ = 5.7 Hz, 2H, -O$CH_2$$\mathbf{CH_2}$N), 2.87 (s, 6H, -N$(\mathbf{CH_3})_2$).

(8e). 1,2-Bis(phenyl)-8-tetrahydropyranyloxy-octanone (3.04 g, 8.0 mmol) in 30 mL dry THF was added dropwise to the Grignard solution and stir the reaction mixture until it forms a paste like material that is not easily soluble in THF. Evaporate THF carefully with a Buchi rotary evaporator. To work up, add 50 mL saturated NH_4Cl to dissolve most of the paste. Extract the product with diethylether (4 × 30 mL) and dried the ethereal solution with $MgSO_4$. Removal of ether yielded dark brown residue (5.3 g).

Mass(m/e), $C_{35}H_{47}O_4N_1$, Average FW = 545.76, LRMS; $[M + H]^+$ 546.3 (100), HRMS; $C_{35}H_{48}O_4N_1$ $[M + H]^+$ (100), Calc. Mass 546.3583, Found 546.3569, 546.3577, 546.3583.

7,8,8-Tris(phenyl)-7-octen-1-ol (9a). A solution of the ketone 1,2-bis(phenyl)-8-tetrahydropyranyloxy-octanone (1.1g, 2.9 mmol) in dry ether was treated with excess of the Grignard reagent, phenylmagnesium bromide (PhMgBr, 18 mmol) for 4h under nitrogen at room temperature and was then hydrolyzed with 50 mL of 10 % aqueous ammonium chloride (aq. NH_4Cl). A cold-water bath was put on to prevent the reaction mixture from the unexpected splash initiated by the heat generated during the neutralization. The ether phase was washed with water (4 × 50 mL), dried and evaporated to give the crude tertiary alcohol intermediate. The product was confirmed by ^{1}H NMR and TLC (Rf = 0.93, hexane:ethylacetate, 8:2). The oily residue was dehydrated and deprotected in 100 mL toluene in the presence of PTSA (100 mg, 0.53 mmol) at reflux for 3 hr. After evaporation of the solvent, the residue was applied to a flash column preequilibrated with hexane:acetone (7:1). The product was eluted from the fraction number 8 to 20 (15 mL/ fraction). The product pool then was collected and the solvent was evaporated. A total of 0.8 g (2.25 mmol) light yellow oil was obtained (Rf = 0.39, hexane:ethylacetate, 8:2). Yield is 77.6 % based on the amount of ketone used. ^{1}H NMR in $CDCl_3$, δ, 6.88 – 7.37 (m, 15H, Ar-**H**), 3.56 (t, $^3J_{HH}$ = 6.7 Hz, 2H, -$\mathbf{CH_2}$OH), 2.44 (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C$\mathbf{CH_2}$), 1.46 (mq, $^3J_{HH}$ = 6.7 Hz, 2H, -$\mathbf{CH_2}CH_2$OH), 1.35 (bs, 3H), 1.23 (bm, 5H,). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 143.7, 143.2, 142.6, 141.1, 139.3, 130.9, 129.8, 129.7, 128.3, 128.0, 127.6, 126.8, 126.3, 125.9, 63.2, 35.9, 32.8, 29.6, 28.9, 25.5. Mass (m/e), $C_{26}H_{28}O_1$, Average FW = 356.51, LRMS; $[M - H]^-$ 355.4 (100) HRMS; $C_{26}H_{28}O_1$ $[M - H]^-$ (100), Calc. Mass 355.2062, Found 355.2051.

7,8-Bis(phenyl)-7-(4-methoxyphenyl)-7-octen-1-ol (9b). Typically, a Grignard was prepared from 460 mg (18.9 mmol) of magnesium (Mg) and 2.98 g (2 mL, 15.9 mmol) of 4-bromoanisole in the presence of a crystal of iodine (I_2) in 100 mL of dry diethylether in a glove box. The Grignard reagent was usually ready after stirring at room temperature overnight (16 h). A solution of the ketone 1,2-bis(phenyl)-8-tetrahydropyranyloxy-octanone (2.34 g, 6.16 mmol) in dry ether was treated with excess of the Grignard reagent, methoxyphenylmagnesium bromide (CH_3OPhMgBr, 18.9 mmol) for 4h under nitrogen at room temperature and was then hydrolyzed with 50 mL of 10% aqueous ammonium chloride (aq. NH_4Cl). A cold-water bath was put on to prevent the reaction mixture from the unexpected splash initiated by the heat generated during the neutralization. The ether phase was washed with water (4 × 50 mL), dried and evaporated to give the crude tertiary alcohol intermediate. The product was confirmed by ^{1}H NMR and TLC (Rf = 0.40, hexane:ethylacetate, 8:2). The oily residue was dehydrated and deprotected in 100 mL of 95 % ethanol in the presence of PPTS (100 mg, 0.34 mmol) at reflux for 3 hr. After evaporation of the solvent, the residue was applied to a flash column preequilibrated with hexane:acetone (7:1). The product was eluted from the fraction number 8 to 20 (15 mL/ fraction). The product pool then was collected and the solvent was evaporated. A total of 1.79 g (4.63 mmol) light yellow oil was obtained (Rf = 0.21, hexane:ethylacetate, 8:2). Yield is 75.2 % based on the amount of ketone used. ^{1}H NMR in $CDCl_3$, δ, 6.55 – 7.36 (E/Z) (m, 20H, 2 × Ar-**H**(B,C)), 6.78 (Z) (d, $^3J_{HH}$ = 4.4 Hz, 2H, o-**H**-Ar(A),), 6.55 (Z) (d, $^3J_{HH}$ = 4.4 Hz, 2H, m-**H**-Ar(A)), 3.84 (E) (s, 3H, -O$\mathbf{CH_3}$), 3.69 (Z) (s, 3H, -O$\mathbf{CH_3}$), 3.57 (t, $^3J_{HH}$ = 6.5 Hz, 2H, -$\mathbf{CH_2}$OH), 2.48 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C$\mathbf{CH_2}$), 2.43 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C$\mathbf{CH_2}$), 1.44 – 1.49 (E/Z) (m,

4H, 2 × -$\mathbf{CH_2}$$CH_2OH$), 1.33 – 1.37 (E/Z) (m, 4H, 2 × C=CCH$_2$**CH$_2$**-), 1.21 – 1.28 (E/Z) (m, 8H, 4 × –**CH$_2$**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 158.4, 157.6, 144.0, 143.5, 142.9, 140.8, 140.2, 138.7, 136.1, 135.6, 132.0, 130.8, 129.6, 128.0, 127.5, 126.2, 125.5, 113.6, 130.9, 129.7, 128.3, 127.9, 126.7, 126.8, 112.9, 63.2, 55.4, 55.1, 36.0, 32.8, 29.6, 28.9, 25.5. Mass(*m/e*), $C_{27}H_{30}O_2$, Average FW = 386.53, LRMS; 387.1 $[M + H]^+$ (100), HRMS(APCI); $C_{27}H_{31}O_2$ $[M + H]^+$ (100), Calc. Mass 387.2324, Found 387.2368.

7,8-Bis(4-methoxyphenyl)-7-(phenyl)-7-octen-1-ol (9c). A solution of the ketone 1,2-bis(4-methoxyphenyl)-8-tetrahydropyranyloxy-octanone (2.34 g, 6.16 mmol) in dry ether was treated with excess of the Grignard reagent, phenylmagnesium bromide (PhMgBr, 18.9 mmol) for 4h under nitrogen at room temperature and was then hydrolyzed with 50 mL of 10% aqueous ammonium chloride (aq. NH_4Cl). A cold-water bath was put on to prevent the reaction mixture from the unexpected splash initiated by the heat generated during the neutralization. The ether phase was washed with water (4 × 50 mL), dried and evaporated to give the crude tertiary alcohol intermediate. The product was confirmed by ^1H NMR and TLC (Rf = 0.93, hexane:ethylacetate, 8:2). The oily residue was dehydrated and deprotected in 100 mL of 95 % ethanol in the presence of PPTS (100 mg, 0.34 mmol) at reflux for 3 hr. After evaporation of the solvent, the residue was applied to a flash column preequilibrated with hexane:acetone (7:1). The product was eluted from the fraction number 8 to 20 (15 mL/ fraction). The product pool then was collected and the solvent was evaporated. A total of 1.79 g (4.63 mmol) light yellow oil was obtained (Rf = 0.18, hexane:ethylacetate, 8:2). Yield is 75.2 % based on the amount of ketone used. ^1H NMR in $CDCl_3$, δ, 6.56 – 7.36 (E/Z) (m, 26H, Ar-**H**), 3.83, 3.76 (E) (s, 6H, 2 × -O**CH$_3$**), 3.78, 3.70 (Z) (s, 6H, 2 × -O**CH$_3$**), 3.57 (E,Z) (t, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × -**CH$_2$**OH), 2.43 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C**CH$_2$**), 2.38 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C**CH$_2$**), 1.47 (E/Z) (bm, 4H, 2 × -**CH$_2$**CH$_2$OH), 1.33 (E/Z) (bm, 4H, 2 × C=CCH$_2$**CH$_2$**-), 1.22 (E/Z) (b, 8H, 4 × –**CH$_2$**-). Mass(*m/e*), $C_{28}H_{32}O_3$, Average FW = 416.56, LRMS(APCI); $[M + H]^+$ 417.1 (100), HRMS; $C_{28}H_{32}O_3$ $[M + H]^+$ (100), Calc. Mass 417.2430, Found 417.2444.

7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-octen-1-ol (9e). Tertiary alcohol Grignard intermediate (280 mg, 0.53 mmol) was dissolved in 30 mL MeOH and 0.5 mL of concentrated hydrochloric acid (HCl) was added. The reaction mixture was heated (70°C) for 2 hrs. After the reaction was completed, most of the solvent was evaporated. Add 30 mL water then 10 mL of 10 % NaOH to ensure the neutralization. The basic solution was washed with diethylether (3 × 30 mL) to extract the product. Ethereal phase was dried with Na_2SO_4 for 5 hrs. Ether was evaporated and the product obtained. (0.25 g, 0.56 mmol). Rf values is 0.59 for the Z-isomer and 0.50 for the E-isomer with solvents acetone:triethylamine(Et_3N), 20:1. E isomer, ^1H NMR in $CDCl_3$, δ, 6.85-7.15 (m, 14H, Ar-**H**), 4.08 (t, $^3J_{HH}$ = 5.9 Hz, 2H, -O**CH$_2$**CH$_2$N), 3.54 (t, $^3J_{HH}$ = 6.6 Hz, 2H, -**CH$_2$**OH), 2.74 (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OCH$_2$**CH$_2$**N), 2.43 (q, $^3J_{HH}$ = 7.9 Hz, 2H, -C=C**CH$_2$**-), 2.35 (s, 6H, -N(**CH$_3$**)$_2$), 1.45 (bt, $^3J_{HH}$ = 6.3 Hz, 2H, -**CH$_2$**CH$_2$OH), 1.34 (bt, 2H, - **CH$_2$**CH$_2$CH$_2$OH), 1.22 (bs, 4H, 2 × -**CH$_2$**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 157.5, 143.2, 142.6, 140.6, 138.6, 135.9, 130.6, 130.5, 129.5, 127.7, 127.2, 125.9, 125.5, 114.0, 65.9, 62.8, 58.3, 45.9, 35.7, 32.5, 29.3, 28.7, 25.2. Mass(*m/e*), $C_{30}H_{37}O_2N_1$, Average FW = 443.63, LRMS; 444.3 $[M + H]^+$ (100), HRMS; $C_{30}H_{38}O_2N_1$ $[M + H]^+$ (100), Calc. Mass 444.2903, Found 444.2909. Z isomer, ^1H NMR in $CDCl_3$, δ, 6.56-7.37 (m, 14H, Ar-**H**), 3.92 (t, $^3J_{HH}$ = 5.8 Hz, 2H, -O**CH$_2$**CH$_2$N), 3.52 (t, $^3J_{HH}$ = 6.7 Hz, 2H, -**CH$_2$**OH), 2.64 (t,

$^3J_{HH}$ = 5.8 Hz, 2H, -OCH$_2$**CH$_2$**N), 2.41 (q, $^3J_{HH}$ = 7.9 Hz, 2H, -C=C**CH$_2$**-), 2.28 (s, 6H, -N(**CH$_3$**)$_2$), 1.44 (t, $^3J_{HH}$ = 6.6 Hz, 2H, -**CH$_2$**CH$_2$OH), 1.33 (t, $^3J_{HH}$ = 7.5 Hz, 2H, -**CH$_2$**CH$_2$CH$_2$OH), 1.21 (bs, 4H, 2 × -**CH$_2$**-). ^{13}C{^{1}H} NMR in CDCl$_3$, δ, 156.9, 144.0, 142.8, 140.2, 138.7, 135.7, 132.0, 129.7, 129.7, 128.3, 128.0, 126.7, 126.2, 113.6, 65.8, 63.0, 58.5, 46.1, 36.0, 32.8, 29.6, 29.0, 25.5. Mass(*m/e*), $C_{30}H_{37}O_2N_1$, Average FW = 443.63, LRMS; 444.3 [M + H]$^+$ (100), HRMS; $C_{30}H_{38}O_2N_1$ [M + H]$^+$ (100), Calc. Mass 444.2903, Found 444.2909.

1-bromo-7,8,8-tris(phenyl)-7-octene (10a). A solution of the alcohol, 7,8,8-tris(phenyl)-7-octen-1-ol (**9a**, 661 mg, 1.86 mmol), carbon tetrabromide (CBr$_4$, 2.46 g, 7.44 mmol), and triphenylphosphine (PPh$_3$, 1.95 g, 7.44 mmol) in dry ether (100 mL) was stirred at room temperature for 12 h under a nitrogen (N$_2$) atmosphere. The brown precipitate was filtered off using a Whatman filter paper. The resulting solution was washed with water (4 × 30 mL), dried with MgSO$_4$, and evaporated to a pale yellow oily liquid. The product was purified from a flash column chromatography (hexane:acetone, 95:5). The bromo compound eluted in the early fractions. (Rf = 0.87, hexane:ethylacetate, 8:2). ^{1}H NMR in CDCl$_3$, δ, 6.89 – 7.71 (m, 15H, Ar-**H**), 3.33 (t, $^3J_{HH}$ = 7.5 Hz, 2H, -**CH$_2$**Br), 2.45 (t, $^3J_{HH}$ = 7.8 Hz, 2H, C=C**CH$_2$**), 1.74 (m, $^3J_{HH}$ = 6.9 Hz, 2H, -**CH$_2$**CH$_2$Br), 1.21 – 1.38 (m, 6H, 3 × –**CH$_2$**-). ^{13}C{^{1}H} NMR in CDCl$_3$, δ, 143.6, 143.1, 142.5, 141.0, 139.4, 130.9, 129.7, 129.6, 128.3, 128.0, 127.5, 126.8, 126.4, 125.9, 35.8, 34.1, 32.8, 28.9, 28.7, 28.0.

1-Bromo-7,8-bis(phenyl)-8-(4-methoxyphenyl)-7-octene (10b). A solution of the alcohol, 7,8-bis(phenyl)-8-(4-methoxyphenyl)-7-octen-1-ol (1.79 g, 4.63 mmol), carbon tetrabromide (CBr$_4$, 6.14 g, 18.5 mmol), and triphenylphosphine (PPh$_3$, 4.86 g, 18.5 mmol) in dry ether (100 mL) was stirred at room temperature for 12 h under a nitrogen (N$_2$) atmosphere. The brown precipitate was filtered off using a Whatman filter paper. The resulting solution was washed with water (4 × 30 mL), dried with MgSO$_4$, and evaporated to a pale yellow oily liquid. The product was purified from a flash column chromatography (hexane:acetone, 95:5). The final product (1.82 g, 4.0 mmol, 86.4 % yield) eluted in the early fractions. (Rf = 0.61, hexane:ethylacetate, 8:2). ^{1}H NMR in CDCl$_3$, δ, 6.55 – 7.37 (E/Z) (m, 28H, 2 × Ar-**H**), 3.84 (E) (s, 3H, O**CH$_3$**), 3.68 (Z) (s, 3H, O**CH$_3$**), 3.33 (E,Z) (t, $^3J_{HH}$ = 6.7 Hz, 4H, 2 × -**CH$_2$**Br), 2.42 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C**CH$_2$**), 2.42 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=C**CH$_2$**), 1.72 – 1.79 (E/Z) (m, 4H, 2 × -**CH$_2$**CH$_2$Br), 1.22 – 1.38 (E/Z) (m, 12H, 6 × –**CH$_2$**-). ^{13}C{^{1}H} NMR in CDCl$_3$, δ, 158.5 (157.7), 144.0, 143.5 (142.8), 140.7 (140.1), 139.0 (138.8), 136.1 (135.6), 132.0, 130.9 (130.8), 129.8 (129.7), 128.3 (128.1), 128.0, 127.5, 126.8 (126.3), 125.9, 113.7 (113.0), 55.4 (55.2), 35.9, 34.1, 32.8, 29.0, 28.9, 28.0. Mass(*m/e*), $C_{27}H_{29}O_1Br_1$, Average FW = 449.43, LRMS(APCI); [M]$^+$ 449.1 (100), 451.0 (97), HRMS; Cald for [M + Na]$^+$ (100), $C_{27}H_{29}O_1Br_1$ 417.2430, Found 417.2444.

1-Bromo-7,8-bis(4-methoxyphenyl)-8-(phenyl)-7-octene (10c). A solution of the alcohol, 7,8-bis(4-methoxyphenyl)-8-(phenyl)-7-octen-1-ol (5.75 g, 13.8 mmol), carbon tetrabromide (CBr$_4$, 18.3 g, 55.2 mmol), and triphenylphosphine (PPh$_3$, 14.5 g, 55.2 mmol) in dry ether (100 mL) was stirred at room temperature for 12 h under a nitrogen (N$_2$) atmosphere. The brown precipitate was filtered off using a Whatman filter paper. The resulting solution was washed with water (4 × 30 mL), dried with MgSO$_4$, and evaporated to a pale yellow oily liquid. The product was purified from a flash column chromatography (hexane:acetone, 95:5). The final product (5.50 g, 11.5 mmol,

83.2 % yield) eluted in the early fractions. ^{1}H NMR in $CDCl_3$, δ, 6.55 – 7.34 (E/Z) (m, 26H, 2 × Ar-**H**), 3.83, 3.75 (E) (2 × s, 6H, 2 × OC**H$_3$**), 3.77, 3.69 (Z) (2 × s, 6H, 2 × OC**H$_3$**), 3.34 (E, Z) (t, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × -C**H$_2$**Br), 2.43 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CC**H$_2$**), 2.38 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CC**H$_2$**), 1.71 – 1.78 (E/Z) (m, 4H, $^3J_{HH}$ = 7.4 Hz, 2 × -C**H$_2$**CH$_2$Br), 1.20 – 1.32 (E/Z) (m, 12H, 6 × –C**H$_2$**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 158.0, 157.5, 144.2, 139.5, 138.3, 135.8, 134.9, 132.0, 130.8, 129.7, 128.3, 126.6, 113.4, 113.0, 55.3, 55.8, 35.9, 34.1, 32.8, 29.0, 28.9, 28.0. Mass(*m/e*): $C_{28}H_{31}O_2Br_1$, Average FW = 479.46, HRMS (TOF MS ES$^+$); $[M]^{+\cdot}$ $C_{28}H_{31}O_2Br_1$ Cald. Mass 478.1507, Found 478.1507, $[M + Na]^+$ $C_{28}H_{31}O_2Na_1{}^{81}Br_1$ Cald. Mass 503.1385, Found 503.1382.

1-Bromo-7,8-bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octene (10e). 7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-octen-1-ol (264 mg, 0.59 mmol) was prepared in 20 mL methylenechloride (CH_2Cl_2) in a 50 mL round bottomed flask. The bromination reaction was initiated by addition of excess phosphorus tribromide (PBr_3, 1 mL, 10.5 mmol) and the reaction mixture was refluxed for 24 hrs in N_2 environment. During the reaction, bright orange precipitates formed. The reaction was quenched by the addition of 20 mL MeOH and reflux the mixture for 30 minutes. Remove the solvent by a Buchi rotary evaporator. Add 40 mL 10 % sodium hydroxide (NaOH) solution to the residue and the product was extracted with 50 mL diethylether. The ethereal layer was washed with saturated potassium chloride (KCl, 3 × 30 mL) solution. The ethereal phase was dried with anhydrous $MgSO_4$. Removal of $MaSO_4$ and evaporation of diethylether afforded the product (173 mg, 0.34 mmol, 57.6 % yield). Rf values is 0.74 with acetone:triethylamine (Et_3N), 20:1. ^{1}H NMR in $CDCl_3$, δ 6.86-7.36 (E, Z) (m, 20H, Ar-**H**), 6.77 (d, $^3J_{HH}$ = 8.7 Hz, 2H, m-Ar-**H**), 6.56 (d, $^3J_{HH}$ = 8.7 Hz, 2H, o-Ar-**H**), 4.15 (E) (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OC**H$_2$**CH$_2$N), 3.99 (Z) (t, $^3J_{HH}$ = 5.6 Hz, 2H, -OC**H$_2$**CH$_2$N), 3.33 (E, Z) (q, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × -C**H$_2$**Br), 2.84 (E) (bt, $^3J_{HH}$ = 5.1 Hz, 2H, -OCH$_2$C**H$_2$**N), 2.75 (Z) (b, 2H, -OCH$_2$C**H$_2$**N), 2.47 (E) (b, 2H, -C=CC**H$_2$**-), 2.40 (Z) (b, 2H, -C=CC**H$_2$**-), 2.43 (E) (s, 6H, -N(C**H$_3$**)$_2$), 2.37 (Z) (s, 6H, -N(C**H$_3$**)$_2$), 1.75 (E, Z) (m, $^3J_{HH}$ = 7.3 Hz, 4H, 2 × -C**H$_2$**CH$_2$Br), 1.32 (E,Z) (bs, 12H, 6 × -C**H$_2$**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, (E, Z), δ, 157.7, 156.9, 144.2, 143.5, 142.8, 141.1, 140.5, 139.3, 139.0, 132.9, 132.7, 132.0, 130.9, 130.8, 129.8, 129.7, 128.3, 128.1, 128.0, 127.5, (126.8, 126.3, 125.9 ;1:2:1), 114.3, 113.6, 65.7, 65.4, 58.4, 58.3, 45.9, 45.8, 35.9, 34.2, 32.8, 31.2, (29.0, 28.8), 28.0.

1-Iodo-7,8-bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octene. To a solution of 1-Bromo-7,8-bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octene (173 mg, 0.34 mmol) in 20 mL dry acetone was added sodium iodide (100 mg, 0.66 mmol). The reaction mixture was stirred at 25°C for 5 h. Then most of the solvent was evaporated and the residue was transferred to a 125 mL separatory funnel with ether (50 mL) and saturated sodium bicarbonate ($NaHCO_3$) solution (50 mL). The organic phase was washed with saturated $NaHCO_3$ (3 × 50 mL), dried with $MgSO_4$, filtered, and concentrated to a viscous liquid. The crude iodide (90 mg, 0.16 mmol, 47 % yield) was used as such at the following alkylation step. ^{1}H NMR in $CDCl_3$, δ, 6.86-7.36 (E, Z) (m, 20H, Ar-**H**), 6.77 (d, $^3J_{HH}$ = 8.7 Hz, 2H, m-Ar-**H**), 6.56 (d, $^3J_{HH}$ = 8.7 Hz, 2H, o-Ar-**H**), 4.11 (E) (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OC**H$_2$**CH$_2$N), 3.95 (Z) (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OC**H$_2$**CH$_2$N), 3.11 (E,Z) (q, $^3J_{HH}$ = 6.6 Hz, 4H, 2 × -C**H$_2$**I), 2.78 (E) (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OCH$_2$C**H$_2$**N), 2.68 (Z) (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OCH$_2$C**H$_2$**N), 2.46 (E) (b, $^3J_{HH}$ = 7.9Hz, 2H, -C=CC**H$_2$**-), 2.43 (Z) (t, 2H, -C=CC**H$_2$**-), 2.38 (E) (s, 6H, -N(C**H$_3$**)$_2$), 2.31 (Z) (s, 6H,

-N(CH_3)$_2$), 1.73 (E,Z) (m, $^3J_{HH}$ = 6.9 Hz, 4H, 2 × -CH_2CH_2I), 1.32 (E) (m, $^3J_{HH}$ = 7.9 Hz, 6H, 3 × -CH_2-), 1.32 (Z) (b, 6H, 3 × -CH_2-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, (E, Z), δ, 157.7, 156.9, 143.9, 143.5, 142.8, 140.7, 140.1, 139.0, 138.9, 132.0, 131.8, 131.0, 130.8, 129.8, 129.7, 128.3, 128.1, 128.0, 127.5, (126.8, 126.3, 125.9 ;1:2:1), 114.8, 114.3, 113.6, 66.0, 65.7, 58.5, 58.4, 46.1, 46.0, 35.9, 33.6, 30.3, (28.8, 28.7), 7.4.

1-Bromo-7,8-bis(phenyl)-8-(4-methoxyphenyl)-7-octene (11b). A solution of 1-bromo-7,8-bis(phenyl)-8-(4-hydroxyphenyl)-7-octene (1.0 g, 2.2 mmol) in dry methylene chloride (CH_2Cl_2) was treated with a solution of boron tribromide (BBr_3, 0.5 mL, 2.2 mmol,) at -60°C (dry ice/chloroform($CHCl_3$) mixture), under a nitrogen atmosphere. After the addition, the reaction mixture was equilibrated to the ambient temperature (25°C) and was stirred for overnight (16 h). Before work up, the mixture was refluxed for 2 hrs. The reaction mixture was cooled with an ice bath before adding 25 mL methanol. The resulting solution was concentrated to 1 – 2 mL, treated with saturated sodium bicarbonate ($NaHCO_3$) solution (50 mL), and extracted with ethyl acetate (4 × 50 mL). The organic phase was dried with $MgSO_4$, and filtered. The resulting filtrate was evaporated to yield a dark brown paste. The residue was purified using a flash column chromatography eluted with hexane:acetone (8:2). The product pool was evaporated to give 0.91 g (2.09 mmol, 95 % yield) pure bromo compound. Rf is 0.06 with hexane:ethylacetate, (4:1). ^{1}H NMR in $CDCl_3$, δ, 6.46 – 7.36 (E/Z) (m, 28H, Ar-**H**), 3.34 (E, Z) (q, $^3J_{HH}$ = 6.8 Hz, 4H, 2 × -CH_2Br), 2.46 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CCH_2), 2.41 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CH_2), 1.71 – 1.81 (E/Z) (m, $^3J_{HH}$ = 6.8 Hz, 4H, 2 × -CH_2CH_2Br), 1.20 – 1.40 (E/Z) (m, 12H, 6 × –CH_2-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, (154.5, 153.7), (143.9, 143.4, 142.8), (140.8, 140.2), (138.9, 138.8), (136.2, 135.7), 132.2, 130.9, 129.8, 129.6, 128.8, 128.3, 128.1, 128.0, 127.5, 126.8, 126.3, 125.9, (115.2, 114.5), 35.9, 34.2, 32.8, (28.9, 28.8), 28.0.

1-Bromo-7,8-bis(4-hydroxyphenyl)-8-(phenyl)-7-octene (11c). A solution of 1-bromo-7,8-bis(4-methoxyphenyl)-8-(phenyl)-7-octene (1.38 g, 2.95 mmol) in dry methylene chloride (CH_2Cl_2) was treated with 2.5 equivalent of boron tribromide (BBr_3, 7.38 mmol,) at -60°C (dry ice/chloroform($CHCl_3$) mixture), under a nitrogen atmosphere. After the addition, the reaction mixture was equilibrated to the ambient temperature (25°C) and was stirred for overnight (16 h). Before work up, the mixture was refluxed for 2 hrs. The reaction mixture was cooled with an ice bath before adding 25 mL methanol. The resulting solution was concentrated to 1 – 2 mL, treated with saturated sodium bicarbonate ($NaHCO_3$) solution (50 mL), and extracted with ethyl acetate (4 × 50 mL). The organic phase was dried with $MgSO_4$, and filtered. The resulting filtrate was evaporated to yield a dark brown paste. The residue was purified using a flash column chromatography eluted with hexane:acetone (8:2). The product pool was evaporated to give 725 mg (1.6 mmol, 54.2 % yield) pure bromo compound. Rf is 0.06 with hexane:ethylacetate, (4:1). ^{1}H NMR in $CDCl_3$, δ, 6.47 – 7.36 (E/Z) (m, 26H, 2 × Ar-**H**), 4.82, 4.67 (E) (bs, 2H, 2 × Ar-**OH**), 4.72, 4.61 (Z) (bs, 2H, 2 × Ar-**OH**), 3.34 (E, Z) (q, $^3J_{HH}$ = 6.7 Hz, 4H, -CH_2Br), 2.42 (E) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CCH_2), 2.37 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, C=CH_2), 1.73 – 1.78 (E/Z) (m, $^3J_{HH}$ = 6.8 Hz, 4H, 2 × -CH_2CH_2Br), 1.21 – 1.37 (E/Z) (m, 12H, 6 × –CH_2-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, (154.3, 153.9, 153.5), (144.0, 143.6), (140.2, 139.6), 138.5, 138.4, (136.5, 136.0), 135.1, 132.2, 131.0, 129.6, 128.3, 127.6, 126.7, 125.8, (115.2, 115.1, 115.0), 114.6, (35.8, 35.8), 34.2, 32.8, 28.9, 28.9, 28.0 Mass(*m/e*), $C_{26}H_{27}O_2Br_1$, Average FW = 451.40, LRMS(APCI); $[M + H]^+$

451.1, 453.1, HRMS; $C_{26}H_{27}O_2Br_1$ $[M - H]^-$ (100), Calc. Mass 449.1116, Found 449.1096.

2-(7,8,8-Tris(phenyl)-7-octenyl)malonic acid diethyl ester (12a). To a stirred suspension of sodium hydride (NaH, 42 mg, 1.05 mmol, 60% dispersion in mineral oil) in dry tetrahydrofuran (THF, 20 mL) was added rapidly diethylmalonate ($CH_2(COOCH_2CH_3)_2$, 171 mg, 1.07 mmol). The reaction mixture was heated (50°C) on a heating mantle for 1 hr under nitrogen atmosphere. After cooling, 1-bromo-7,8,8-tris(phenyl)-7-octene (418 mg, 1.0 mmol) was added dropwise. The reaction mixture was stirred at 55 – 60 °C for 4 days until TLC result showed the disappearance of the reactant under the UV light. Most solvent was evaporated and diethyl ether (75 mL) was added. The ethereal phase was washed with water (4 x 30 mL), brine solution (3 × 30 mL), then dried with $MgSO_4$. Filter the drying agent, evaporate ether using a Buchi rotary evaporator. The residue was then applied to a flash column chromatography equilibrated with benzene. The product was eluted from the fraction number 13 to 40. The product pool was collected and solvent was evaporated to give 0.36 g (0.71 mmol, 71 % yield) product. (Rf = 0.4, hexane:ethylacetate, 8:2(v/v)). ^1H NMR in $CDCl_3$, δ, 6.89 – 7.37 (m, 15H, Ar-H), 4.19 (q, $^3J_{HH}$ = 7.2 Hz, 4H, $-(OCH_2CH_3)_2$, 3.27 (t, $^3J_{HH}$ = 7.5 Hz, 1H, $-CH_2CH(COOEt)_2$, 2.43 (q, $^3J_{HH}$ = 7.9 Hz, 2H, $-C{=}CCH_2$-), 1.82 (m, $^3J_{HH}$ = 6.9 Hz, 2H, $-CH_2CH(COOEt)_2$, 1.26 (t, $^3J_{HH}$ = 7.2 Hz, 6H, $(-OCH_2CH_3)_2$, 1.19 – 1.34 (b, 8H, 4 × $-CH_2$-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 169.7, 143.6, 143.2, 142.6, 141.1, 139.3, 130.9, 129.7, 129.6, 128.5, 128.3, 128.0, 127.5, 126.8, 126.3, 125.9, 61.4, 52.2, 35.9, 29.5, 29.0, 28.9, 28.8, 27.4, 14.3. Mass(*m/e*): C33H38O4, Average FW = 498.66, LRMS(ESI); $[M + H]^+$ 499.1 (45), $[M + Na]^+$ 521.3 (100); HRMS(QTOF); $C_{33}H_{39}O_4$,Calc. Mass 499.2848, Found 499.2868.

2-(7,8-Bis(phenyl)-8-(4-hydroxyphenyl)-7-octenyl)malonic acid diethyl ester (12b). ^1H NMR in $CDCl_3$, δ, 6.47 (Z) (d, $^3J_{HH}$ = 8.5 Hz, 2H, m-Ar-**H**(A)), 6.72 (Z) (d, $^3J_{HH}$ = 8.6 Hz, 2H, o-Ar-**H**(A)), 5.29 (E) (s, 1H, Ar-**OH**), 4.76 (Z) (s, 1H, Ar-**OH**), 4.18 (E, Z) (q, $^3J_{HH}$ = 7.0 Hz, 8H, 2 × $(-OC\mathbf{H_2}CH_3)_2$), 3.26 (E, Z) (tq, $^3J_{HH}$ = 7.6 Hz, 2H, 2 × $-CH_2C\mathbf{H}RCOO$), 2.42 (E) (tq, $^3J_{HH}$ = 7.9 Hz, 2H, $-C{=}CC\mathbf{H_2}$-), 2.38 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, $-C{=}CC\mathbf{H_2}$-), 1.80 (E, Z) (b, 4H, 2 × $-C\mathbf{H_2}CHRCOO$), 1.25 (E, Z) (t, $^3J_{HH}$ = 7.2 Hz, 12H, 2 × $-(COOCH_2C\mathbf{H_3})_2$), 1.17 – 1.33 (E, Z) (b, 16H, 8 × $-C\mathbf{H_2}$-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 169.5(2), 154.3, 142.6, 131.9, 130.6, 129.5, 128.0, 127.8, 127.2, 126.4, 125.9, 125.6, 115.0, 61.3(2), 52.0, 35.7, 29.2(2), 28.8, 28.6(2), 28.5, 27.1. Mass(*m/e*), $C_{33}H_{38}O_5$, Average FW = 514.66, LRMS(ESI); $[M + Na]^+$ 537.2 (58), $[M + H]^+$ 515.1 (23), HRMS; (100), $C_{33}H_{38}O_5Na_1$ $[M + Na]^+$ Cald for 537.2617, Found 537.2596.

2-(7,8-Di(4-hydroxyphenyl)-8-phenyl-7-octenyl)malonic acid diethyl ester (12c). To a stirred suspension of excess sodium hydride (NaH, 92.4 mg, 2.31 mmol, 60% dispersion in mineral oil) in dry tetrahydrofuran (THF, 20 mL) was added rapidly diethylmalonate ($CH_2(COOCH_2CH_3)_2$, 369.6 mg, 2.31 mmol). The reaction mixture was heated (50 °C) on a heating mantle for 1 hr under nitrogen atmosphere. After cooling, 1-bromo-7,8-di(4-hydroxyphenyl)-8-phenyl-7-octene (348 mg, 0.77 mmol) dissolved in 15 mL dry THF was added dropwise. The reaction mixture was stirred at 55 – 60 °C for 2 days until TLC result showed the disappearance of the reactant under the UV light. Most solvent was evaporated and ethyl acetate (75 mL) was added. The organic phase was washed with water (4 × 30 mL), brine solution (3 × 30 mL), then dried with $MgSO_4$. Filter the drying agent; evaporate ethyl acetate using a Buchi rotary evaporator. The

residue was then applied to a flash column chromatography preequilibrated with the eluent (hexane:ethyl acetate, 8:2). The product was eluted from the fraction number 25 to 78 (fraction size, 15 mL/tube). The product pool was collected and the solvent was evaporated to give 0.30 g (0.57 mmol, 74.3 % yield) product. ^{1}H NMR in $CDCl_3$, δ 6.47 - 7.33 (E, Z) (m, 26H, Ar-**H**), 6.31, 6.13, 6.00 (E, Z) (bs, 4H, 4 × Ar-O**H**), 4.18 (E, Z) (q, $^3J_{HH}$ = 7.1 Hz, 8H, 2 × –OC**H$_2$**CH$_3$), 3.30 (E, Z) (tq, $^3J_{HH}$ = 7.4 Hz, 2H, 2 × -CH$_2$C**H**RCOO), 2.39 (E) (tq, $^3J_{HH}$ = 7.9 Hz, 2H, -C=CC**H$_2$**-), 2.35 (Z) (t, $^3J_{HH}$ = 7.9 Hz, 2H, -C=CC**H$_2$**-), 1.82 (E,Z) (b, 4H, 2 × -C**H$_2$**CHRCOO), 1.24 (E, Z) (t, $^3J_{HH}$ = 7.1 Hz, 12H, 2 × –(COOCH$_2$C**H$_3$**)$_2$), 1.14 – 1.35 (E, Z) (b, 16H, 8 × -C**H$_2$**-). $^{13}C\{^1H\}$ NMR in MeOD-d_4, δ, 171.2, 157.2, 156.7, 156.3, 145.7, 145.4, 141.5, 140.7, 139.8, 139.6, 136.5, 136.2, 135.1, 133.1, 132.0, 131.8, 130.7, 129.2, 128.4, 127.5, 126.5, 116.0, 115.8, 115.8, 115.3, 62.5, 53.1, 36.7, 30.4, 29.8, 28.1, 14.5. Mass(*m/e*), $C_{33}H_{38}O_6$, Average FW = 530.66, LRMS (ESI, MeOH); $[M + H]^+$ 531.2 (100), $[M + Na]^+$ 553.3 (100), $[2M + Na]^+$ 1083.9 (67), $[M – H]^-$ 529.1 (100), HRMS; $C_{33}H_{38}O_6Na_1$ $[M + Na]^+$ (100), Calc. Mass 553.2566, Found 553.2559. $C_{33}H_{39}O_6$ $[M + H]^+$ Calc. Mass 531.2747, Found 531.2755.

2-(Dimethylamino)ethyl iodide hydrochloride ($(CH_3)_2NCH_2CH_2I{\cdot}HCl$). To a solution of 2-(dimethylamino)ethyl chloride hydrochloride $(CH_3)_2NCH_2CH_2Cl{\cdot}HCl$, 5 g, 34.7 mmol) in 100 mL dry acetone was added sodium iodide (7.5 g, 52 mmol). The reaction mixture was stirred at 25°C for 5 h. The formation of the iodo compound was confirmed by ^{1}H NMR. The crude iodide x (100 % conversion) was used as such at the following alkylation step. ^{1}H NMR in $CDCl_3$, δ 3.23 (t, $^3J_{HH}$ = 7.4 Hz, 2H, C**H$_2$**I), 2.71 (t, $^3J_{HH}$ = 7.4 Hz, 2H, C**H$_2$**N), 2.32 (s, 6H, N(C**H$_3$**)$_2$). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 61.9, 45.0, 2.5. (Mass(*m/e*), $C_4H_{10}N_1I_1$, Average FW = 199.03, LRMS; $[M + H]^+$ 200.1 (100), $[M – HN(CH_3)_2]^+$ 155, HRMS; $C_{32}H_{40}N_1O_2$ $[M + H]^+$, Calc. Mass 470.3059, Found 470.3050.

2-(7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octenyl)malonic acid diethyl ester (13a). In a solution of 2-(7,8-bis(phenyl)-8-(4-hydroxyphenyl)-7-octenyl)malonic acid diethyl ester (200 mg, 0.39 mmol) dissolved in 5 mL DMF, were added 2-(dimethylamino)ethyl chloride hydrochloride ($(CH_3)_2NCH_2CH_2Cl{\cdot}HCl$, 1.8 eq., 101.1 mg, 0.70 mmol) and potassium carbonate (K_2CO_3, 1 g). The reaction mixture was heated at 80°C with stirring for overnight (16 h). The color of the solution turns to dark orange. After the completion filter the remaining solid residue and evaporate the solvent. Add 30 mL saturated sodium bicarbonate ($NaHCO_3$) solution to the oily residue and transfer the solution to a 125 mL separatory funnel. Extract the product with 30 mL diethylether. The ethereal layer was washed twice with 30 mL saturated sodium bicarbonate solution. After separation, the ethereal phase was dried with anhydrous $MgSO_4$. Removal of $MaSO_4$ and evaporation of diethylether afforded the product (200 mg, 0.34 mmol, 88 % yield). E isomer, ^{1}H NMR in $CDCl_3$, δ, 6.85 - 7.15 (m, 14H, Ar-**H**), 4.18 (q, $^3J_{HH}$ = 7.0 Hz, 4H, 2 × –OC**H$_2$**CH$_3$ and b, 2H, -OC**H$_2$**CH$_2$N), 3.26 (t, $^3J_{HH}$ = 7.0 Hz, 1H, -CH$_2$C**H**RCOO), 2.87 (b, 2H, -OCH$_2$C**H$_2$**N), 2.45 (bs, 6H, -N(C**H$_3$**)$_2$ and 2H, -C=CC**H$_2$**-), 1.81 (b, 2H, -C**H$_2$**CHRCOO), 1.25 (t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 × –OCH$_2$C**H$_3$**), 1.20 (b, 8H, 4 × -C**H$_2$**-). $^{13}C\{^1H\}$ NMR in $CDCl_3$, δ, 169.8, 157.5, 143.5, 142.8, 140.9, 138.8, 136.4, 131.0, 130.8, 129.8, 128.0, 127.5, 126.2, 125.8, 114.3, 65.6, 61.4, 58.3, 52.2, 45.8, 36.1, 29.6, 29.1, 29.0, 28.9, 27.4, 14.3. Mass(*m/e*), $C_{37}H_{47}O_5N_1$, Average FW = 585.78, LRMS(ESI); 586.4 $[M + H]^+$ (100), HRMS; $C_{37}H_{48}O_5N_1$ $[M + H]^+$ (100), Calc. Mass 586.3532, Found 586.3506.

2-(7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octenyl)malonic acid diethyl ester (13a, Z isomer). ^{1}H NMR in $CDCl_3$, δ 7.09 – 7.36 (m, 10H, [B], [C] Ar-**H**), 6.76 (d, $^3J_{HH}$ = 4.4 Hz, 2H, o-**H**-Ar(A)), 6.55 (d, $^3J_{HH}$ = 4.4 Hz, 2H, m-**H**-Ar(A)), 4.18 (q, $^3J_{HH}$ = 7.1 Hz, 4H, 2 × –OC**H$_2$**CH$_3$), 3.95 (t, $^3J_{HH}$ = 5.7 Hz, 2H, -OC**H$_2$**CH$_2$N), 3.25 (t, $^3J_{HH}$ = 7.6 Hz, 1H, -CH$_2$C**H**RCOO), 2.68 (t, $^3J_{HH}$ = 5.6 Hz, 2H, -OCH$_2$C**H$_2$**N), 2.37 (t, $^3J_{HH}$ = 8.0 Hz, 2H, -C=CC**H$_2$**-), 2.32 (s, 6H, -N(C**H$_3$**)$_2$), 1.80 – 1.84 (b, 2H, -C**H$_2$**CHRCOO), 1.23 (t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 × –OCH$_2$C**H$_3$**), 1.18 – 1.23 (b, 8H, 4 × -C**H$_2$**-).

2-(7,8,8-Tris(phenyl)-7-octenyl)malonic acid dipotassium salt (K2-09, 14a). 2-(7,8,8-Tris(phenyl)-7-octenyl)malonic acid diethyl ester (0.36 g, 0.71 mmol) was placed in a 25 mL round bottom flask and was dissolved with 20 mL absolute ethanol. Grinded potassium hydroxide (100 mg, 1.8 mmol) was added. The mixture was refluxed for 2 hrs. White product precipitated and filtered. The product was dried under vacuum and sealed tightly to minimize the contact with water in the air. Yield was 314 mg (85.2 %).

^{1}H NMR in MeOD-*d*$_4$, δ, 6.85 – 7.37 (m, 15H, Ar-**H**), 3.04 (t, $^3J_{HH}$ = 7.6 Hz, 1H, -C**H**(COOK)$_2$), 2.42 (t, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H$_2$**-), 1.73 (bm, $^3J_{HH}$ = 7.3 Hz, 2H, -C**H$_2$**CH(COO-)$_2$), 1.12 – 1.36 (bm, 8H, 4 × -C**H$_2$**-). ^{13}C{^{1}H} NMR in MeOD-*d*$_4$, δ, 181.0, 145.0, 144.6, 143.9, 142.6, 140.7, 131.9, 130.9, 130.6, 129.4, 129.0, 128.5, 127.9, 127.3, 126.9, 60.5, 37.0, 32.5, 31.1, 30.9, 30.2, 30.0. ^{13}C{^{1}H} NMR in MeOD-*d*$_4$ 4 days later, δ, 181.0, 145.0, 144.6, 143.9, 142.5, 140.7, 131.9, 130.9, 130.6, 129.4, 129.0, 128.5, 127.8, 127.3, 126.9, 37.0, 32.4, 31.1, 30.9, 30.2, 30.0.

2-(7,8-Bis(phenyl)-8-(4-hydroxyphenyl)-7-octenyl)malonic acid dipotassium salt (K2-10, 14b, E isomer). 2-(7,8-Bis(phenyl)-8-(4-hydroxyphenyl)-7-octenyl)malonic acid diethyl ester (81.4 mg, 0.16 mmol) was placed in a 25 mL round bottom flask and was dissolved with 20 mL absolute ethanol. Grinded potassium hydroxide (17.7 mg, 0.32 mmol) was added. The mixture was refluxed for 2 hrs. The reaction progress was monitored by ^{1}H NMR. An appearance of the characteristic triplet (or overlap of triplet due to the coexistence of the E, Z isomer) of the C-2 carbon of the malonato group at 3.0 ppm confirms the completion of the reaction. The reaction solution was filtered and the filtrate was dried under vacuum. The dried residue was extracted with methanol (3 × 10 mL) and the methanol pool was filtered and evaporated to recover the salt. ^{1}H NMR in MeOD-*d*$_4$, (E isomer), δ, 6.85 (d, $^3J_{HH}$ = 8.6 Hz, 2H, m-Ar-**H** (B)), 6.71 (d, $^3J_{HH}$ = 8.6 Hz, 2H, o-Ar-**H** (B)), 6.91 – 7.54 (m, 10H, Ar-**H**), 3.05 (t, $^3J_{HH}$ = 7.5 Hz, 1H, -C**H**(COOK)$_2$), 2.43 (b, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H$_2$**-), 1.76 (bm, $^3J_{HH}$ = 7.3 Hz, 2H, -C**H$_2$**CH(COOK)$_2$), 1.20 – 1.29 (2 × bs, 8H, 4 × -C**H$_2$**-). ^{13}C{^{1}H} NMR in MeOD-*d*$_4$, (E isomer), δ, 181.2, 157.6, 145.1, 144.4, 141.9, 140.6, 136.1, 132.0, 131.8, 130.9, 128.9, 128.4, 127.1, 126.7, 116.1, 37.1, 32.8, 31.1, 30.9, 30.8, 30.2, 30.0. Mass(*m/e*): $C_{29}H_{28}O_5K_2$, Average FW = 534.73, LRMS(ESI); $[M + H]^+$ 535.1 (100), $[M + K]^+$ 573.1 (25), $[M + H_3O]^+$ 553.1 (15), $[2M + H]^+$ 1069.1 (100), $[2M + K]^+$ 1107.1 (26), $[M - 2K + H]^-$ 457.2, $[M - 2K - CO_2 + H]^-$ 413.5 (100), HRMS(TOF-MS ES$^+$); $C_{29}H_{29}O_5K_2$ $[M + H]^+$ Cald for 535.1289, Found 535.1292, $[M + H_3O]^+$ 553.2413; $[M + K]^+$ Cald for 573.0871, Found 573.0861.

2-(7,8-Di(4-hydroxyphenyl)-8-phenyl-7-octenyl)malonic acid dipotassium salt (K2-11, 14c). 2-(7,8-Di(4-hydroxyphenyl)-8-phenyl-7-octenyl)malonic acid diethyl ester (103 mg, 0.2 mmol) was placed in a 25 mL round bottom flask and was dissolved with 20 mL absolute ethanol. Grinded potassium hydroxide (50 mg, 0.9 mmol) was added. The

mixture was refluxed for 2 hrs. The reaction progress was monitored by ^{1}H NMR. An appearance of the characteristic triplet (or overlap of triplet due to the coexistence of the E, Z isomer) of the C-2 carbon of the malonato group at 3.0 ppm confirms the completion of the reaction. The reaction solution was filtered and the filtrate was dried under vacuum. The dried residue was extracted with methanol (3 × 10 mL) and the methanol pool was filtered and evaporated to recover the salt. Yield was 94 mg (85.4 %).
^{1}H NMR in MeOD-d_4, δ, 6.38 – 7.33 (E, Z) (m, 26H, Ar-**H**), 3.04 (E, Z) (t, $^3J_{HH}$ = 7.5 Hz, 2H, 2 × -C**H**(COOK)$_2$), 2.40 (E) (t, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H**$_2$-), 2.32 (Z) (t, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H**$_2$-), 1.73 (E, Z) (bm, 4H, 2 × -C**H**$_2$CH(COOK)$_2$), 1.11 – 1.40 (E, Z) (2 × bs, 16H, 2 × 4 × -C**H**$_2$-). ^{1}H NMR in MeOD-d_4 (after 1 day, triplet at C-2 is missing, exchanged with solvent), δ, 6.41 – 7.33 (E, Z) (m, 26H, Ar-**H**), 2.40 (E) (b, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H**$_2$-), 2.33 (Z) (b, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H**$_2$-), 1.73 (E, Z) (bm, 4H, 2 × -C**H**$_2$CD(COOK)$_2$), 1.13 – 1.35 (E, Z) (2 × bs, 16H, 2 × 4 × -C**H**$_2$-). ^{13}C{^{1}H} NMR in MeOD-d_4, δ, 181.1, 161.6, 146.5, 146.3, 141.5, 140.2, 139.4, 139.3, 134.0(2), 133.1, 132.2, 131.8, 130.8, 129.0, 128.1, 127.2, 126.0, 117.7, 117.4, 117.3, 116.9, 60.7, 37.0, 32.5, 31.3, 31.3, 31.1, 30.5, 30.1. Mass(*m/e*), $C_{29}H_{28}O_6K_2$, Average FW. = 550.73, LRMS(ESI); $[M + H]^+$ 551.1, $[M + K]^+$ 589.1, $[2M - K + H]^+$ 1063.0 (100), $[2M + H]^+$ 1100.9 (70), $[M - 2K + H]^-$ 473.1 (50), $[M - 2K - CO_2 + H]^-$ 429.5 (100), HRMS(TOF-MS ES$^+$); $C_{29}H_{29}O_6K_2$ $[M + H]^+$ (100) Cald. 551.1238, Found 551.1261, $[M + K]^+$ (60) Found 589.0809.

Diammine[2-(7,7,8-triphenyl-7-octenyl)malonato]platinum(II) (Pt-09, 15a). 2-(7,8,8-Tris(phenyl)-7-octenyl)malonic acid dipotassium salt (31.8 mg, 61.4 μmol) was dissolved with 2 mL H_2O in a 25 mL round bottom flask. Weigh equivalent amount of cisdiamminedichloro platinum (II) (cis-DDP, 18.4 mg, 61.3 μmol), then dissolved with 3 mL dimethylformamide (DMF). The cis-DDP/DMF solution was added to the malonate salt solution. The reaction mixture was heated at 60 °C with stirring. A bluish precipitate formed and filtered off. To the filtrate, add more DMF (2 mL) and H_2O (1 mL). The mixture was heated at 60 °C with stirring for overnight (16 h). End point was decided by monitoring the reaction progress with ^{195}Pt NMR of an aliquot (0.5 mL) of the reaction mixture. Off-white precipitate was formed and filtered. The precipitate was washed with diethylether (20 mL) to get rid of residual organic solvent. The product was air dried for several hours. A nice amorphous off-white product was obtained (26 mg, 63.4 % yield based on the amount of the starting salt used). ^{1}H NMR in DMSO-d_6, δ, 6.85 – 7.42 (m, 15H, Ar-**H**), 4.14 (b, 6H, Pt(N**H**$_3$)$_2$), 2.58 (t, $^3J_{HH}$ = 5.2 Hz, 1H, -C**H**(COO-)$_2$), 2.32 (t, $^3J_{HH}$ = 7.7 Hz, 2H, -C=CC**H**$_2$-), 1.64 (bm, $^3J_{HH}$ = 5.6 Hz, 2H, -C**H**$_2$CH(COO-)$_2$), 1.02 – 1.12 (bm, $^3J_{HH}$ = 5.8 Hz, 8H, 4 × -C**H**$_2$-). ^{13}C{^{1}H} NMR in DMSO-d_6, δ, 175.1, 162.6, 143.0, 142.9, 141.9, 140.6, 138.9, 130.2, 129.4, 129.1, 128.5, 128.0, 127.7, 126.9, 126.4, 125.9, 48.7, 35.2, 30.2, 29.0 (2), 28.2, 26.4. ^{195}Pt NMR in DMSO-d_6, δ, -1711 (300 MHz). ^{1}H NMR in DMSO-d_6, δ, 6.85 – 7.42 (m, 15H, Ar-**H**), 4.14 (bs, 6H, Pt(N**H**$_3$)$_2$), 3.46 (t, $^3J_{HH}$ = 7.0 Hz, 1H, -C**H**(COO-)$_2$), 2.36 (t, $^3J_{HH}$ = 7.6 Hz, 2H, -C=CC**H**$_2$-), 1.71 (bm, 2H, -C**H**$_2$CH(COO-)$_2$), 1.11 – 1.24 (bs, 8H, 4 × -C**H**$_2$-). ^{13}C{^{1}H} NMR in DMSO-d_6, δ, 175.9, 142.9, 142.7, 141.8, 140.5, 138.7, 130.1, 129.2, 129.0, 128.3, 127.9, 127.5, 126.7, 126.2, 125.8, 57.8, 35.3, 29.3, 29.1, 28.9, 28.196, 27.5. ^{195}Pt NMR in DMSO-d_6, δ -1715.

Diammine[2-(7,8-diphenyl-7-(4'-hydroxyphenyl)-7-octenyl)malonato]platinum(II) (Pt-10, 15b). 2-(7,8-Diphenyl-8-(4-hydroxyphenyl)-7-octenyl)malonic acid dipotassium salt (140 mg, 0.26 mmol) was dissolved with 2 mL H_2O in a 25 mL round bottom flask. Weigh equivalent amount of cisdiamminedichloro platinum(II) (cis-DDP, 80.0 mg, 0.27 mmol), then dissolved with 4 mL dimethylformamide (DMF). The cisDDP/DMF solution was added to the malonate salt solution. The pH of the solution was adjusted to pH 6.5. The reaction mixture was heated at 65 °C with stirring. A dark precipitate formed and filtered off. To the filtrate, add more DMF (2 mL) and H_2O (1 mL). The mixture was heated at 65 °C with stirring for overnight (16 h). End point was decided by monitoring the reaction progress with ^{195}Pt NMR of an aliquot (0.5 mL) of the reaction mixture. The reaction mixture was filtered and the solvent evaporated. The residue was washed with D_2O and diethylether (20 mL) to get rid of residual organic solvent. The residue was extracted with 10 mL MeOH and filtered again. After evaporation of the MeOH light yellowish green product was obtained (54.5 mg, 0.074 mmol, 31.5 % yield based on the amount of the starting salt used). $^{13}C\{^1H\}$ NMR in MeOD-d_4, δ, 181.1, 165.2, 145.9, 144.9, 141,6, 140.5, 132.2, 131.8, 131.6, 131.0, 129.6, 128.8, 128.7, 128.2, 126.9, 126.8, 126.4, 120.1, 118.8, 60.6, 37.2, 32.5, 31.3, 31.1, 30.5, 30.1. ^{195}Pt NMR in MeOD-d_4, δ -1705 (Varian 300). ^{1}H NMR in MeOD-d_4, δ, 6.41 – 7.32 (E, Z) (m, 26H, Ar-**H**), 2.44 (E) (t, $^3J_{HH}$ = 7.6 Hz, 2H, -C=CC**H$_2$**-), 2.37 (Z) (t, $^3J_{HH}$ = 7.7 Hz, 2H, -C=CC**H$_2$**-), 1.72 (E, Z) (bm, 4H, 2 × -**CH$_2$**CH(COO-)$_2$), 1.11 – 1.40 (E, Z) (2 × bs, 16H, 2 × 4 × -**CH$_2$**-). $^{13}C\{^1H\}$ NMR in MeOD-d_4, δ, 181.0, 157.3, 156.5, 145.3, 144.9, 144.2, 141.8, 141.0, 140.3, 136.1, 135.7, 133.0, 131.8, 131.7, 130.8, 130.5, 129.3, 128.9, 128.9, 128.3, 127.6, 127.1, 126.6, 116.1, 115.3, 36.9, 32.2, 30.9(2), 30.8, 30.7, 30.1, 30.0, 29.8, 28.9. Mass(*m/e*), $C_{29}H_{34}O_5N_2Pt_1$, Average FW = 685.21, LRMS(ESI); [M - H]$^-$ 684.2 (53), [M + K - H]$^-$ 722.1 (16) ^{195}Pt NMR in MeOD-d_4, δ, -1707.29.

Diammine[2-(7,8-di(4-hydroxyphenyl)-8-phenyl-7-octenyl)malonato]platinum(II) (Pt-11, 15c). 2-(7,8-Di(4-hydroxyphenyl)-8-phenyl-7-octenyl)malonic acid dipotassium salt (50 mg, 0.09 mmol) was weighed into a small vial and it was dissolved with 3 mL H_2O. Cis-DDP (24.8 mg, 0.083 mmol) was weighed and it was dissolved in 6 mL DMF. Two solutions were mixed together in a 50 mL round bottomed flask. The pH of the reaction mixture was adjusted to 6.5. The solution was heated (65°C) with stirring for 3 days under nitrogen (N_2) atmosphere. Occasionally an aliquot (0.5 mL) was subjected to the ^{195}Pt NMR to check the reaction progress. After the reaction, the mixture was filtered to remove the insoluble dark byproducts. Solvent was evaporated with vacuum. The product was extracted with 40 mL MeOH. The methanol solution was filtered and the filtrated was evaporated. Light greenish yellow product obtained (30.8 mg, 0.044 mmol, 53 % yield). ^{1}H NMR in Acetone-d_6, δ, 6.50 – 7.36 (E, Z) (m, 26H, Ar-**H**), 3.23 (E, Z) (t, $^3J_{HH}$ = 7.0 Hz, 2H, 2 × -C**H**(COO-)$_2$), 2.43 (E) (t, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H$_2$**-), 2.36 (Z) (t, $^3J_{HH}$ = 7.8 Hz, 2H, -C=CC**H$_2$**-), 1.81 (E, Z) (bm, 4H, 2 × -**CH$_2$**CH(COO-)$_2$), 1.16 – 1.40 (E, Z) (2 × bs, 16H, 2 × 4 × -**CH$_2$**-). ^{195}Pt NMR in MeOD-d_4, δ, -1705.

2-(7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octenyl)malonic acid disodium salt (Na2-CCN, 16a). 2-(7,8-Bis(phenyl)-8-[4-2(dimethylamino)ethoxy]phenyl-7-octenyl)malonic acid diethyl ester (178 mg, 0.35 mmol) was placed in a 25 mL round bottom flask and was dissolved with 20 mL

methanol (MeOH). Grinded sodium hydroxide (NaOH, 2.5 eq., 35 mg, 0.88 mmol) was added. The mixture was refluxed for 2 hrs. The reaction progress was monitored by ^{1}H NMR. An appearance of the characteristic triplet (or overlap of triplet due to the coexistence of the E,Z isomer) of the C-2 carbon of the malonato group at 3.0 ppm confirms the completion of the reaction. The reaction solution was filtered and the filtrate was dried under vacuum. The dried residue was extracted with methanol (3 × 10 mL) and the methanol pool was filtered and evaporated to recover the salt (144 mg, 0.25 mmol, 72 %). ^{1}H NMR in MeOD-d_4, δ, 6.56-7.35 (m, 28H, Ar-**H**) (E, Z), 4.13 (t, $^3J_{HH}$ = 5.5 Hz, 2H, -OC**H$_2$**CH$_2$N) (E), 3.96 (t, $^3J_{HH}$ = 5.5 Hz, 2H, -OC**H$_2$**CH$_2$N) (Z), 3.05 (t, $^3J_{HH}$ = 7.6 Hz, 2H, 2 × -CH$_2$C**H**RCOO) (E,Z), 2.79 (t, $^3J_{HH}$ = 5.3 Hz, 2H, -OCH$_2$C**H$_2$**N) (E), 2.68 (t, $^3J_{HH}$ = 5.5 Hz, 2H, -OCH$_2$C**H$_2$**N) (Z), 2.37 – 2.45 (q, t, 4H, 2 × -C=CC**H$_2$**-) (E, Z), 2.35 (s, 6H, -N(C**H$_3$**)$_2$) (E), 2.28 (s, 6H, -N(C**H$_3$**)$_2$) (Z), 1.73 (b, 4H, 2 × -C**H$_2$**CHRCOO) (E, Z), 1.29 (b, 8H, 4 × -C**H$_2$**-) (E), 1.11 (b, 8H, 4 × -C**H$_2$**-) (Z). ^{13}C{^{1}H} NMR in MeOD-d_4, δ, 181.2, 114.6 – 145.2, 66.8, 66.6, 60.8, 59.2, 46.0, 37.0, 32.9, 31.3, 31.0, 30.3, 29.9. Mass(*m/e*), $C_{33}H_{37}O_5N_1Na_2$, Average FW = 573, LRMS (VG 7070-HF, FAB); [M + H]$^+$ 574.3 (48), [M + Na]$^+$ 596.2 (95), HRMS (VG70E-HF, FAB); $C_{33}H_{37}O_5N_1Na_2$ [M + H]$^+$ (48), Calc. Mass 572.2545, Found 574.2553. [M + Na]$^+$ (95) Calc. Mass 596.2365, Found 596.2358.

(Pt-CCN, 17a). Mass(*m/e*), $C_{33}H_{43}O_5N_3Pt_1Na_1$ Average FW = 779.8, LRMS (VG 7070-HF, FAB); [M + H]$^+$ 779.3 (multiplicity), HRMS (VG70E-HF, FAB); $C_{33}H_{43}O_5N_3{}^{194}Pt_1Na_1$, Found 778.27272 (22.19), $C_{33}H_{43}O_5N_3{}^{195}Pt_1Na_1$, Found 779.27483 (22.80), $C_{33}H_{43}O_5N_3{}^{196}Pt_1Na_1$, Found 780.27499 (17.18), $C_{32}{}^{13}CH_{43}O_5N_3{}^{196}Pt_1Na_1$, Found 781.27834 (6.35).

(2) Growth Inhibition Study

Results and discussion

The *in vitro* study of the platinum conjugates against several cancer cell lines is described. As always is the case, testing a drug to a target cell is the first and the fundamental step in the long process of the novel drug development. Choosing the right cell lines is important in order to insure that their interaction with the new drug is the true representative of the targeted design. A proper control cell line may or may not be necessary depending on the purpose of the experiment, however, it would be very difficult task to establish a real control because of the very complex nature of a living cell. Also, a very easy and useful detection method is needed to generate meaningful data in a timely fashion.

In order to address the anticancer effect of the novel drugs, we selected four different cell lines: One (MCF-7) from the breast cancer cell line and the remaining three, PA-1, OVCAR-3, and SK-OV-3, are from the ovarian cancer cell lines. Currently OVCAR-3, MCF-7 and SK-OV-3 are confirmed to carry the estrogen receptor, therefore these cell lines are considered as ER-positive (+). PA-1 is chosen because of its easy handling and its quick response time to the drug test, and can be used as a control to determine the effect of new drug in this study regardless of the status of ER in this cell line.

(a) <u>Comparison of the conjugates</u>. Different conjugates were evaluated on the growth of MCF-7 breast cancer cell line. A typical plot showing the cell survival at different concentrations of the conjugates is shown in **Figure 1**. None of the conjugates

examined here is as potent as carboplatin. However, the tamoxifen portion has quite substantial effect on the potency of the conjugates. It appears that the potency does not correlate with the estrogen receptor binding affinity, although it needs to be confirmed by in vitro measurement of the binding constant.

Analogous study was also performed on OVCAR-3, SK-OV-3, and PA-1 ovarian cancer cell line. The cell survival vs concentrations plots are shown in **Figure 2-4**. None of the conjugates examined here is as potent as carboplatin. SK-OV-3 behaves analogously as MCF-7. In contrast with MCF-7 and SK-OV-3, the potency on OVCAR-3 shows quite nice correlation with the estimated estrogen receptor binding affinity.

(b) Origin of cytotoxicity. In order to determine whether the conjugates dissociate in the medium before entering the cell, comparison of the conjugates with their corresponding potassium or sodium salts was conducted on OVCAR-3 ovarian cancer cell line, as shown in **Figure 5-9**. The conjugates are always much more potent than their corresponding alkaline salts, independent of cancer cell lines. This suggests that the conjugates do not dissociate into tamoxifen dicarboxylate salt and platinum aqueous species.

(c) Synergistic effect. It is interesting to determine whether synergistic effect exists among different fragments of the conjugates. Pt-CCN, Na2-CCN, carboplatin, and tamoxifen were examined on PA-1 ovarian cancer cell lines. Their survival vs concentrations plot is shown in **Figure 10**. Tamoxifen is the most potent drug among the examined. For the first time, the conjugate Pt-CCN is more potent than carboplatin. This means that no synergistic effect is observed among the components of the conjugates. However, the tamoxifen moiety increases the potency of the platinum portion.

(d) Correlation. It is always interesting to observe correlations of potency among different cancer cell lines. The IC_{50} values of the conjugates and some platinum complexes on MCF-7 and SK-OV-3 show nice correlation, as shown in **Figure 11**. The exact cause for this is unclear at this time.

Experimental

(a) Materials. The cancer cell lines, MCF-7 (Breast cancer), PA-1, OVCAR-3, and SK-OV-3 (Ovarian cancer) are from the American Type Culture Collection (ATCC) (Rockville, MD). The MCF-7 cell line was originated from the Michigan Cancer Foundation. The PA-1 and OVCAR-3 cell lines were established by National Institute of Health. The SK-OV-3 cell line was from the Memorial Sloan-Kettering Cancer Center. The cell growth media RPMI-1640 and L-glutamine (gln) were from BioWhittaker. Trypsin-Versene mixture solution was also from BioWhittaker. T-25 (25 mL), T-75 (75 mL) monolayer cell culture flasks were from Falcon. Fetal bovine serum (FBS) was purchased from the Sigma Chemical Co. (St. Louis, MO). Tamoxifen citrate salt (Min. 99 %) ((Z)-1-(p-Dimethylaminoethoxyphenyl)-1,2-diphenyl-1-butene) was from the Sigma Chemical Co. The control platinum based antineoplastic drug carboplatin (DCBDA, diammine(1,1-cyclobutanedicarboxylato)platinum(II), 98 %) was purchased from the Aldrich Chemical Co. (Milwakee, WI). The Methylthiazoletetrazolium (MTT, app. 98 %) and dimethyl sulfoxide (DMSO) for the cell viability was also obtained from the Sigma Chemical Co. The cell culture grade 96-well plates were from Corning. Cell culture was handled in a sterile environment by using either a SterilGARD Hood (Model SG-600) or a SterilGardII (ClassII, Type A/B 3) from the BAKER COMPANY, Inc.

(Sanford, ME). Cell culture were incubated at 37°C in a sterile environment by using either a 5 % CO_2 incubator model FS-11 from the Fisher Scientific or a NUAIRE™ IR AUTOFLOW CO_2 Water-Jacketed Incubator with a constant flow of 5.0 % CO_2. Cell count was performed by using a COULTER® MULTISIZER IIE equipped with a computer and a sampling STAND model IIA from COULTER COORPORATION. A tabletop BECKMAN MODEL GS-6R centrifuge was from Beckman Company.

(b) Cell culture. The breast cancer cell line, MCF-7 (Estrogen receptor (ER) positive(+)), and the ovarian cancer cell lines, PA-1 (wild type [wt] p53), OVCAR-3 (Estrogen receptor(ER) positive (+)), and SK-OV-3 (Estrogen receptor(ER) positive(+), p-53 null) were obtained from the ATCC. These monolayer cells were cultured in RPMI-1640 media (BioWhittaker) supplemented with 10 % FBS and 1 % glutamine (gln). Typically, 1.0 mL frozen MCF-7 cell (same procedure for the other cell lines) in a cryovial was thawed and temperature of the vial was equilibrated to 37°C. The cell solution is carefully seeded into a T-25 flask for monolayer cell growth containing 6 mL RPMI-1640 media. After day 1, the old media was replaced by new media if the cell grows well. The cell growth can be monitored under a microscope (x 20). If the cell population reaches up to about 70 % of the space, a passage of the cell line was performed.

After around 10 – 15 passages, cell culture was harvested for the drug test. Before harvest, the growth of the cell culture in a T-75 flask was examined under a microscope. The old media was removed with aspiration by a plastic pipette connected to a vacuum line. Add 3 mL of the Trypsin-Versene solution to the monolayer cell culture to detach cells from the plastic surface. Incubate the Trypsin-Versene treated cell culture at 37°C in a sterile environment. After the cells were detached from the surface cells were subjected to be counted with a COULTER® MULTISIZER IIE. A 10 mL cell/NaCl solution was prepared by adding 200 µL cell to a 9.8 mL saline solution. Cell numbers were counted and 100 µL cells were seeded onto 96 well plastic cell culture plate. Typically the cell concentrations were 1,000 cells/well for PA-1 and OVCAR-3 cell lines and 500 cells/well for MCF-7 and SK-OV-3 cell lines. Before adding any drug, cells were incubated for 1 day for PA-1 and OVCAR-3 cell lines and for 2 days for SK-OV-3 and MCF-7 cell lines to ensure the proper attachment of the cell lines and propagation.

(c) Growth Inhibition Study. Stock solutions of cis-DDP, carboplatin, tamoxifen-citrate, 4-hydroxytamoxifen, K2-09, K2-10, K2-11, Na2-CCN (Potassium or sodium salts), Pt-09, Pt-10, Pt-11, and Pt-CCN (Platinum Complexes) for the growth inhibition study were prepared by dissolving the corresponding drugs in a minimal amount of either DMSO or MeOH (200 µL). A number of serial dilutions was made to prepare the final drug solutions by mixing the stock solutions with RPMI-1640 media supplemented with 10 % FBS and 2 mM L-glutamnine (L-gln). Add 100 µL drugs in various concentrations into the day 1 (PA-1, OVCAR-3) and the day 2 (MCF-7, SK-OV-3) cell cultures. Incubate the drug-cell mixture for 4 days (PA-1, OVCAR-3) or for 5 days (MCF-7, SK-OV-3) at 37°C in a sterile environment.

(d) MTT assay. MTT assay were performed to establish the anticancer effect of the drugs on the growth of the cancer cell lines PA-1, OVCAR-3, MCF-7, and SK-OV-3. Typically, a fresh stock solution of MTT (Methylthiazoletetrazolium, 4 mg/mL) was prepared with RPMI-1640 media with no supplement. The MTT assay started by adding

25 μL MTT to each well (200 μL) and incubate for 5 hours at 37°C. After incubation, centrifuge the 96 well plates in swinging bucket rotors at 1,110 rpm for 5 minutes using a BECKMAN MODEL GS-6R centrifuge. Drain the supernatants by placing the 96 well plates upside down on paper towels. This allows the integrity of the cell mass as intact as possible. The use of a regular aspirator is very risky for this procedure because of the high possibility of the loss of the light cell mass. DMSO (200 μL) was added into each well and the 96 well plates were allowed to stand for 20 minutes with gentle shaking. The plates were read spectroscopically using a dual-wavelength filter (570 and 630 nm). The concentrations of complexes that decreased absorption by 50 % were calculated by extrapolation and taken as the IC_{50} values.

KEY RESEARCH ACCOMPLISHMENTS

(1) Four conjugates between tamoxifen fragment and platinum moiety have been synthesized and characterized.
(2) The conjugates have been evaluated in a number of breast and ovarian cancer cell lines. All of them show cytotoxicity. The potency depends the cancer cell lines used. For PA-1 ovarian cancer cell line, one conjugate shows higher potency than that of carboplatin.

REPORTABLE OUTCOME

(1) The synthesis and characterization of the conjugates
(2) The anti-tumor and anti-cancer activity on breast and ovarian cancer cell lines.

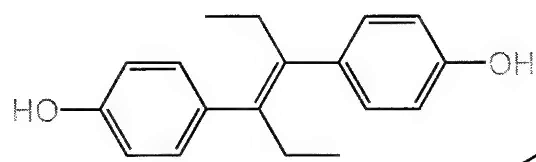
CONCLUSIONS

A number of conjugates between tamoxifen and platinum have been designed, synthesized, and characterized. They all show cytotoxicity. At least one conjugate has shown greater potency than that of carboplatin. The tamoxifen portion has substantial effect on the potency of the drug. However, the change of potency correlates poorly with the estimated estrogen receptor binding affinity. Correlation of potency among different cancer cell lines has been attempted. It is discovered that the IC_{50} values for MCF-7 and SK-OV-3 are correlated.

APPENDICES

(1) 11 Schemes
(2) 11 Figures
(3) A related publication (7 pages)

Reference
[1] Cai, L.; Ren, S.; Cadena, R. S.; Beck, W. T. *J. Med. Chem.* **2001**, *44*, 2959-65. "Synthesis and *in vitro* Antitumor Activity of Oligonucleotide-tethered Platinum Complexes."
[2] Pasini, A.; Caldirola, C. *Inorg. Chim. Acta* **1988**, *151*, 19-20. "A New Synthetic Method for Diaminomalonatoplatinum Type Complexes and the Unexpected Behavior of [$PtCl_2$(trans-dach)]."
[3] Bérubé, G.; He, Y.; Groleau, S.; Séné, A.; Thérien, H. -M.; Caron, M. *Inorg. Chim. Acta* **1997**, *262*, 139-45. "The Synthesis and Characterization of New Triphenylethylene Platinum(II) Complexes."



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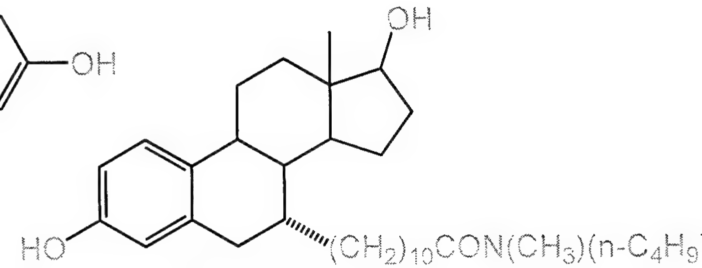

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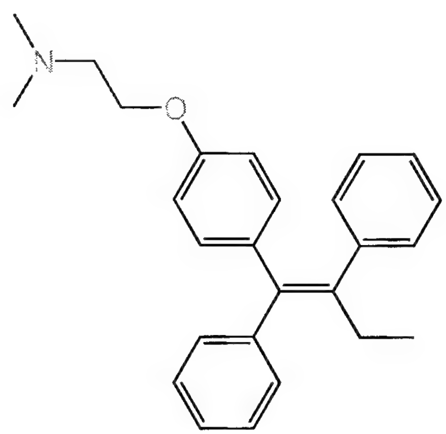
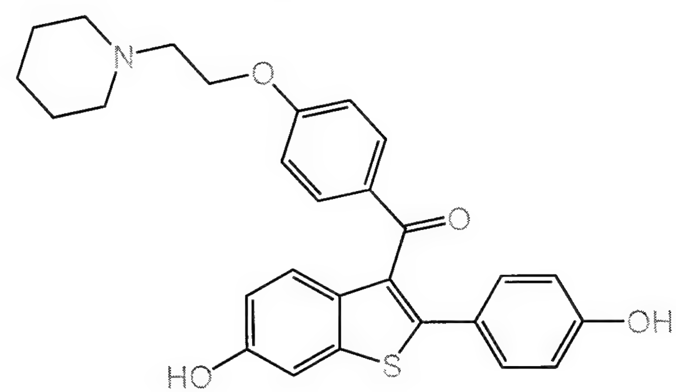
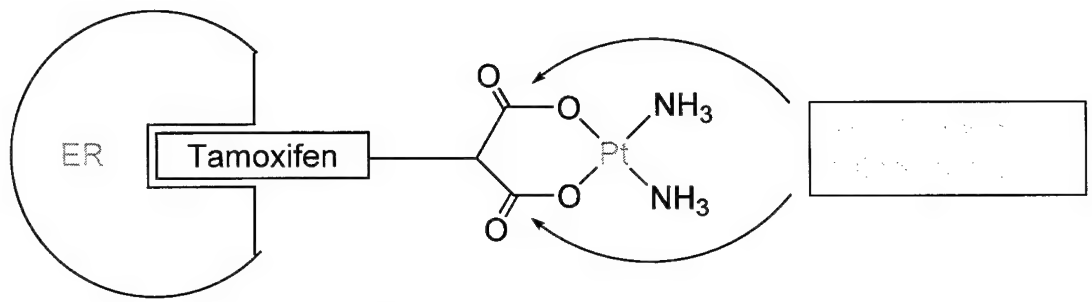


Tamoxifen

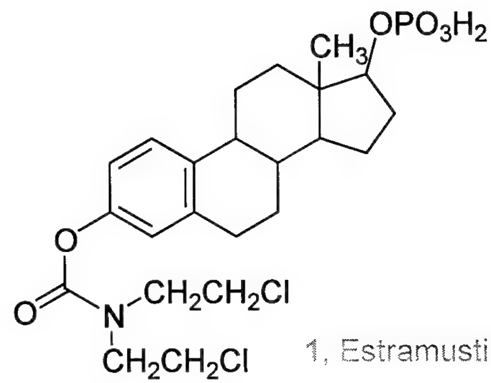


Raloxifene

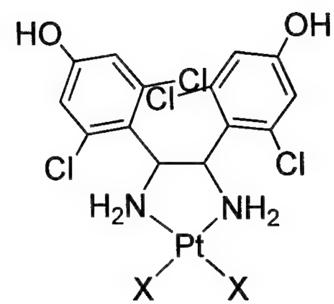
Scheme 1



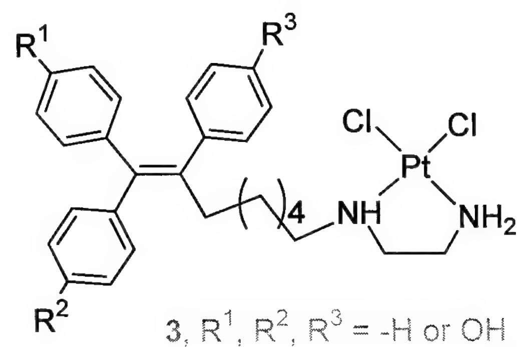
Scheme 2



1, Estramustine



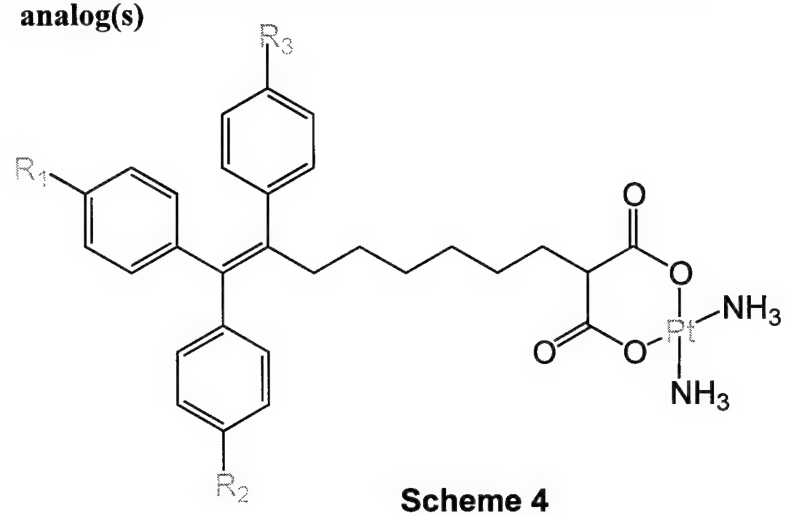
2, X = Cl, 1/2SO_4



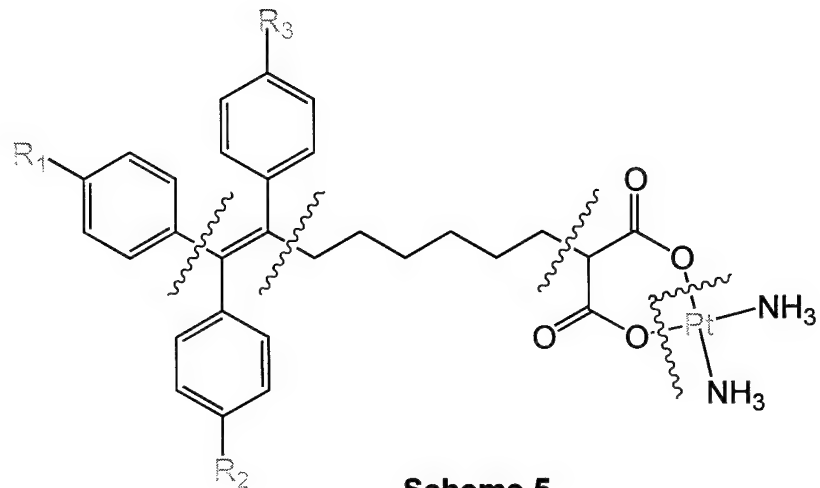
3, R^1, R^2, R^3 = -H or OH

Scheme 3

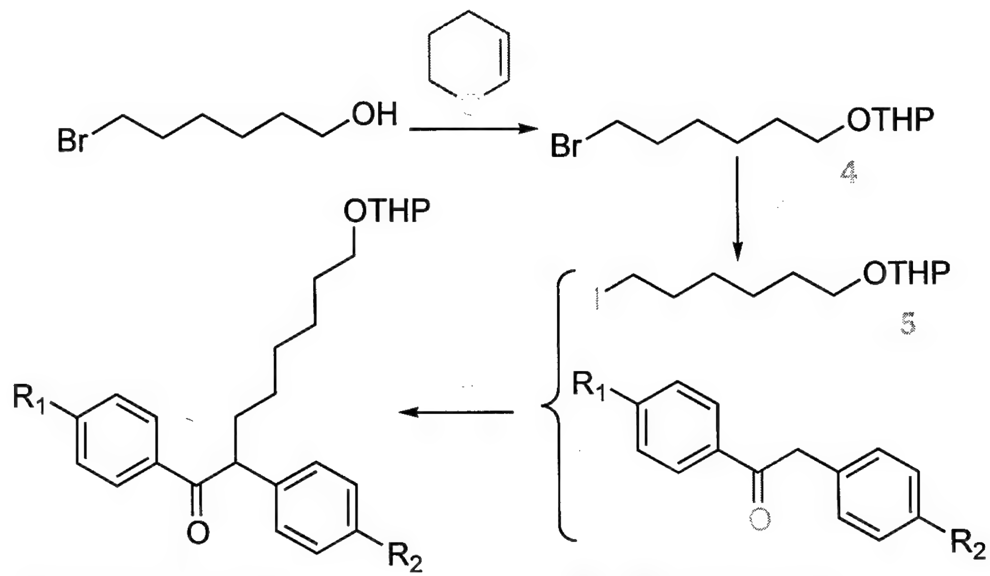
Conjugates of platinum compounds with tamoxifen analog(s)



Scheme 4



Scheme 5

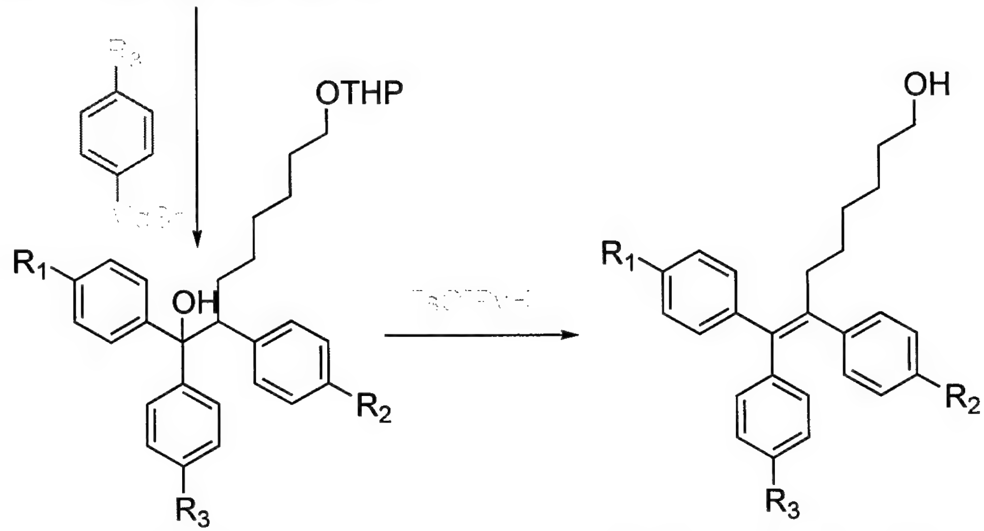


7a, R_1 = H, R_2 = H
7b, R_1 = OCH_3, R_2 = H
7c, R_1 = OCH_3, R_2 = OCH_3

6a, R_1 = H, R_2 = H
6b, R_1 = OCH_3, R_2 = H
6c, R_1 = OCH_3, R_2 = OCH_3

Scheme 6

7a, $R_1 = H, R_2 = H$
7b, $R_1 = OCH_3, R_2 = H$
7c, $R_1 = OCH_3, R_2 = OCH_3$

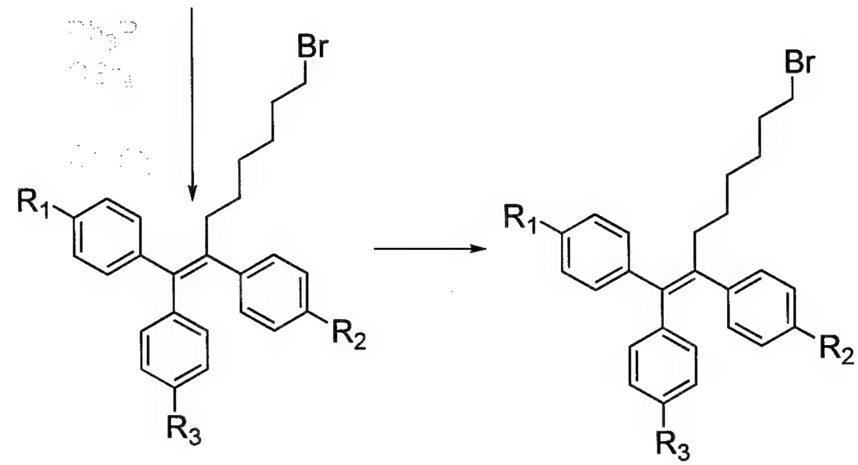


8a, $R_1 = R_2 = R_3 = H$
8b, $R_1 = R_2 = H, R_3 = OCH_3$
8c, $R_1 = OCH_3, R_2 = OCH_3, R_3 = H$
8d, $R_1 = R_2 = R_3 = OCH_3$
8e, $R_1 = R_2 = H, R_3 = OCH_2CH_2N(CH_3)_2$

9a, $R_1 = R_2 = R_3 = H$
9b, $R_1 = R_2 = H, R_3 = OCH_3$
9c, $R_1 = OCH_3, R_2 = OCH_3, R_3 = H$
9d, $R_1 = R_2 = R_3 = OCH_3$
9e, $R_1 = R_2 = H, R_3 = OCH_2CH_2N(CH_3)_2$

Scheme 7

9a, $R_1 = R_2 = R_3 = H$
9b, $R_1 = R_2 = H, R_3 = OCH_3$
9c, $R_1 = OCH_3, R_2 = OCH_3, R_3 = H$
9d, $R_1 = R_2 = R_3 = OCH_3$

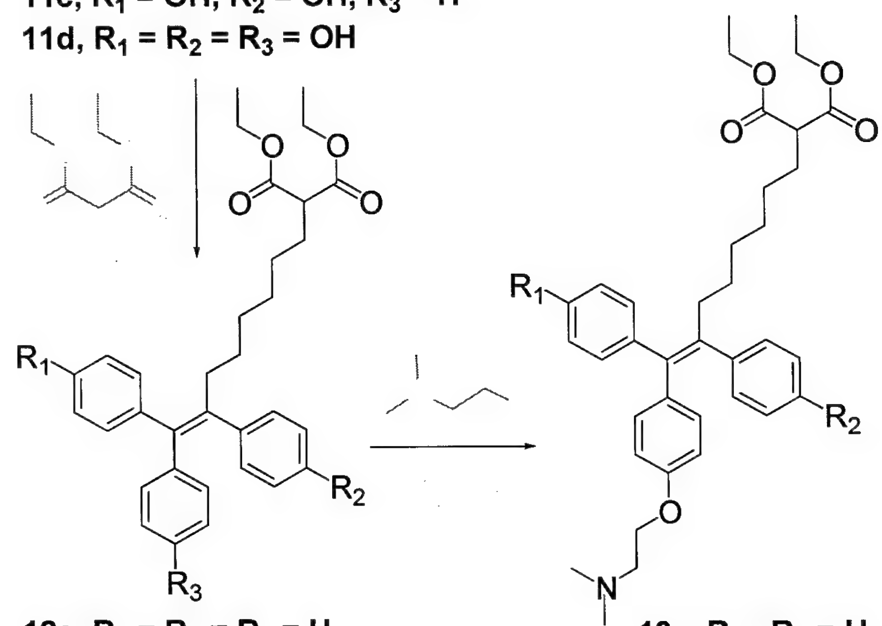


10a, $R_1 = R_2 = R_3 = H$
10b, $R_1 = R_2 = H, R_3 = OCH_3$
10c, $R_1 = OCH_3, R_2 = OCH_3, R_3 = H$
10d, $R_1 = R_2 = R_3 = OCH_3$
10e, $R_1 = R_2 = H, R_3 = OCH_2CH_2N(CH_3)_2$

11a, $R_1 = R_2 = R_3 = H$
11b, $R_1 = R_2 = H, R_3 = OH$
11c, $R_1 = OH, R_2 = OH, R_3 = H$
11d, $R_1 = R_2 = R_3 = OH$

Scheme 8

11a, $R_1 = R_2 = R_3 = H$
11b, $R_1 = R_2 = H, R_3 = OH$
11c, $R_1 = OH, R_2 = OH, R_3 = H$
11d, $R_1 = R_2 = R_3 = OH$

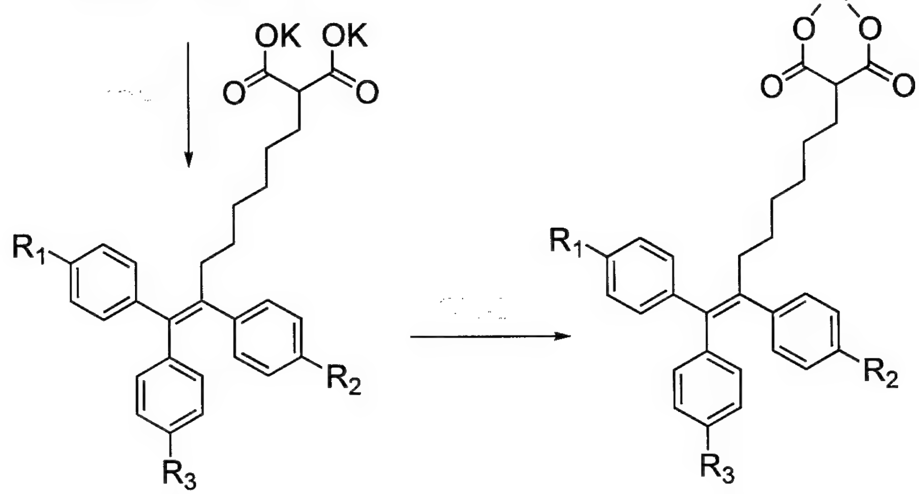


12a, $R_1 = R_2 = R_3 = H$
12b, $R_1 = R_2 = H, R_3 = OH$
12c, $R_1 = OH, R_2 = OH, R_3 = H$
12d, $R_1 = R_2 = R_3 = OH$

13a, $R_1 = R_2 = H$

Scheme 9

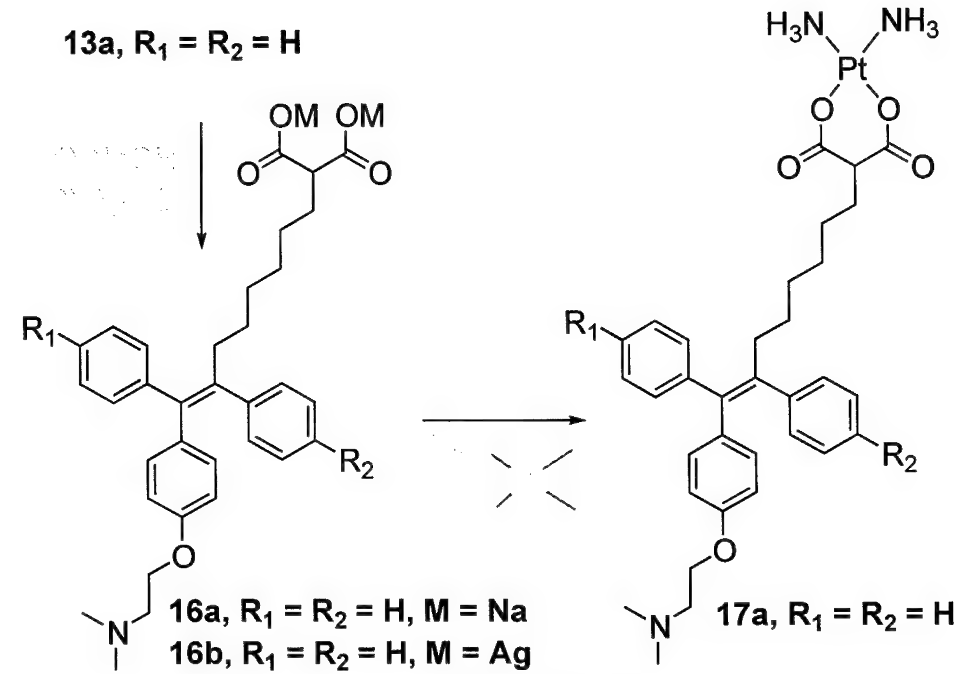
12a, $R_1 = R_2 = R_3 = H$
12b, $R_1 = R_2 = H, R_3 = OH$
12c, $R_1 = OH, R_2 = H, R_3 = OH$
12d, $R_1 = R_2 = R_3 = OH$



14a, $R_1 = R_2 = R_3 = H$
14b, $R_1 = R_2 = H, R_3 = OH$
14c, $R_1 = OH, R_2 = OH, R_3 = H$
14d, $R_1 = R_2 = R_3 = OH$

15a, $R_1 = R_2 = R_3 = H$
15b, $R_1 = R_2 = H, R_3 = OH$
15c, $R_1 = OH, R_2 = OH, R_3 = H$

Scheme 10



13a, $R_1 = R_2 = H$

16a, $R_1 = R_2 = H$, M = Na
16b, $R_1 = R_2 = H$, M = Ag

17a, $R_1 = R_2 = H$

Scheme 11

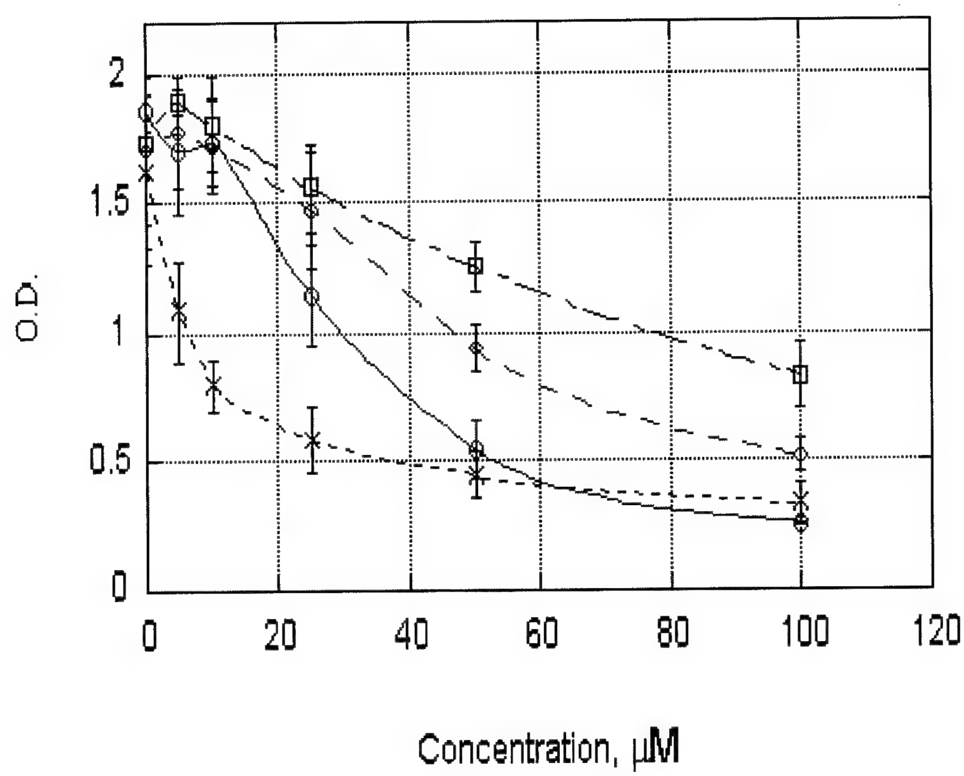


Figure 1 Effect of the conjugates on the growth of the MCF-7 breast cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce cell survival by 50%. Pt-09 (circle), Pt-10 (rectangle), Pt-11 (diamond), carboplatin (cross).

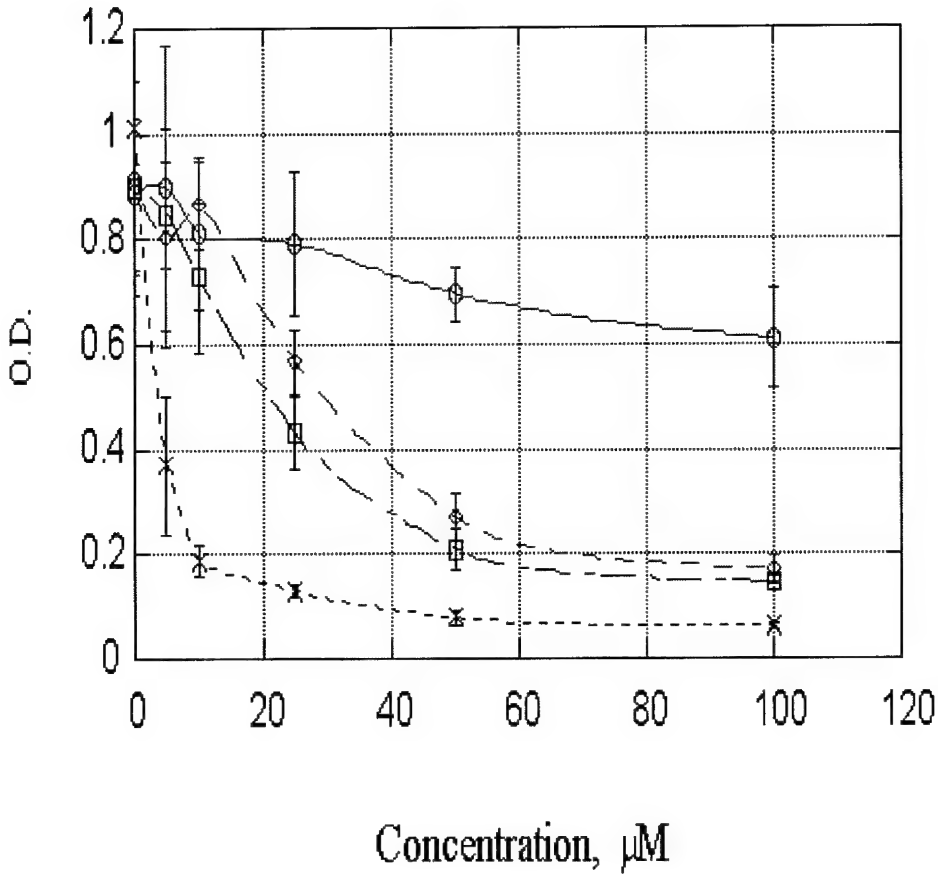


Figure 2. Effect of the conjugates on the growth of the OVCAR-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-09 (circle), Pt-10 (rectangle), Pt-11 (diamond), carboplatin (cross).

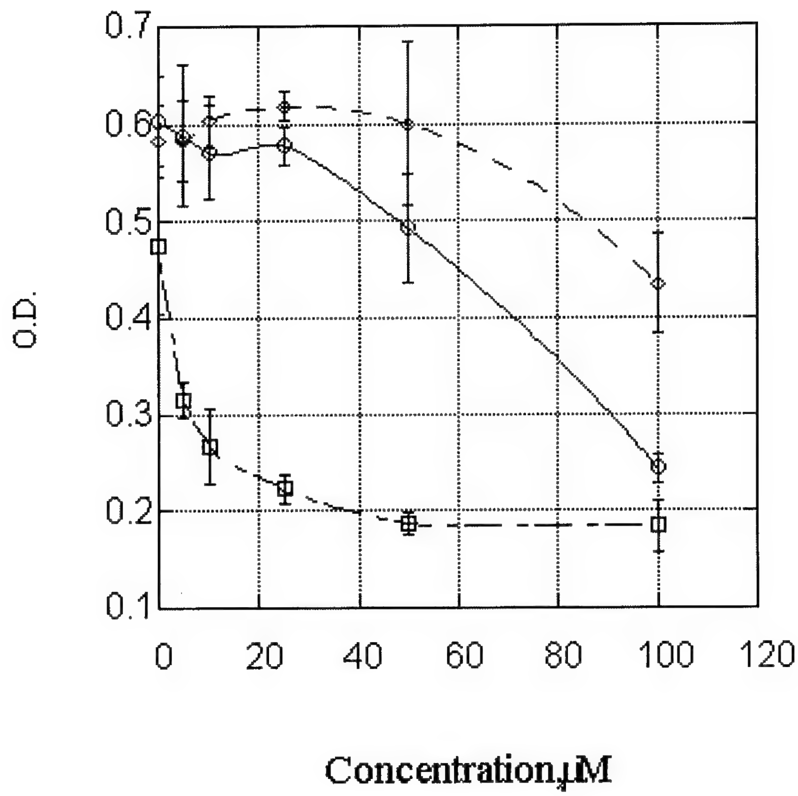


Figure 3. Effect of Pt-09 complex and K2-09 salt on the growth of the OVCAR-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-09 (circle), K2-09 (diamond), carboplatin (rectangle).

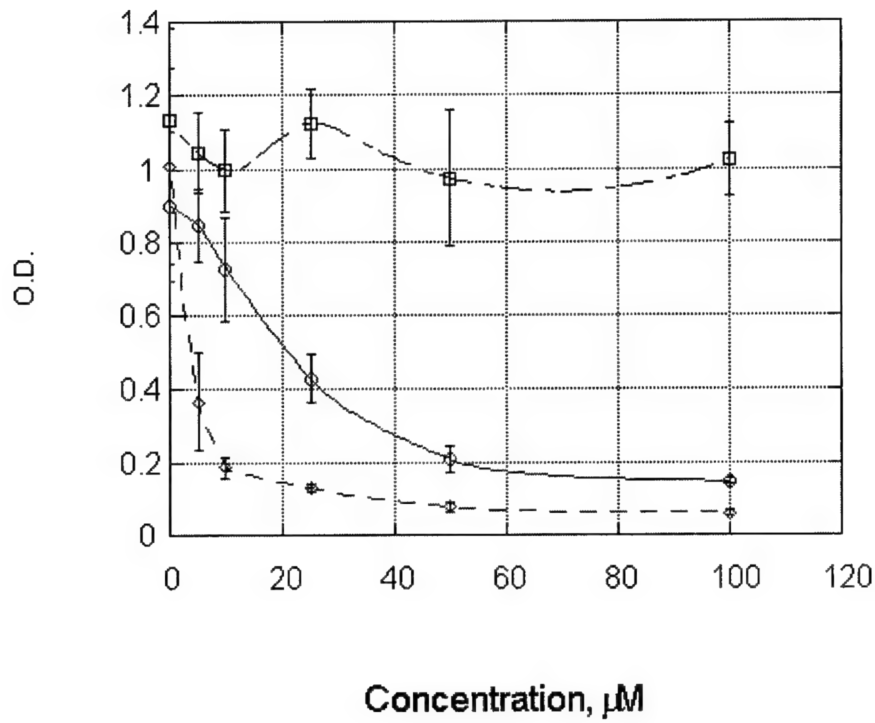


Figure 4. Effect of Pt-10 complex and K2-10 salt on the growth of the OVCAR-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-10 (circle), K2-10 (rectangle), carboplatin (diamond).

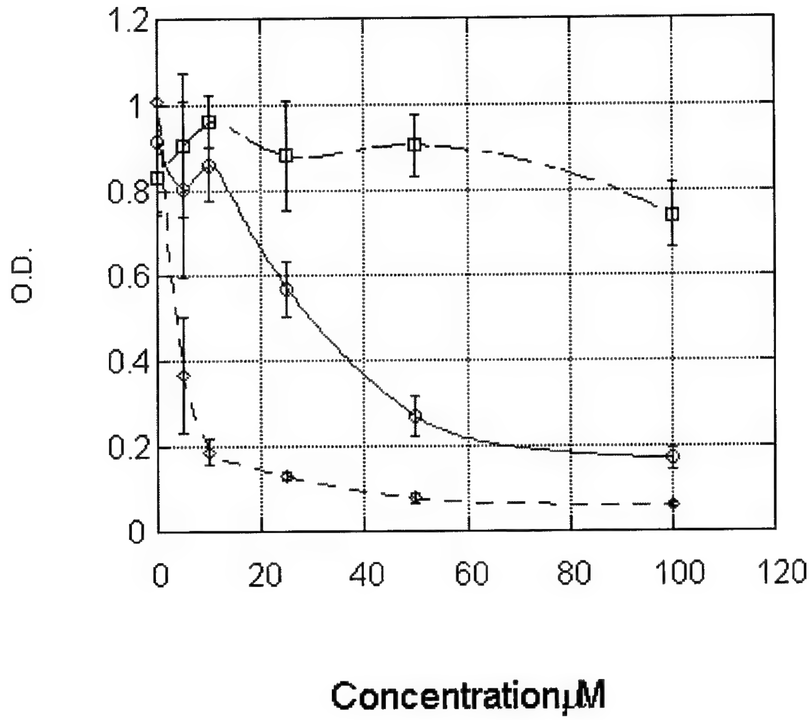


Figure 5. Effect of Pt-11 complex and K2-11 salt on the growth of the OVCAR-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-11 (circle), K2-11 (rectangle), carboplatin (diamond).

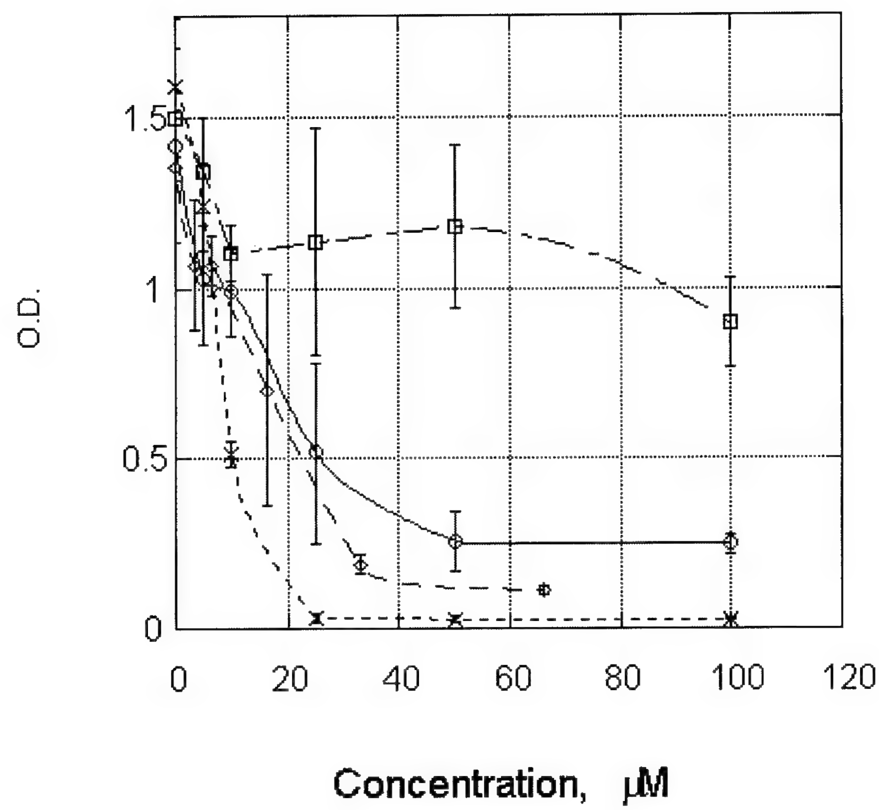


Figure 6. Effect of Pt-CCN on the growth of the PA-1 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-CCN (diamond), Na2-CCN (rectangle), carboplatin (circle), and tamoxifen (cross).

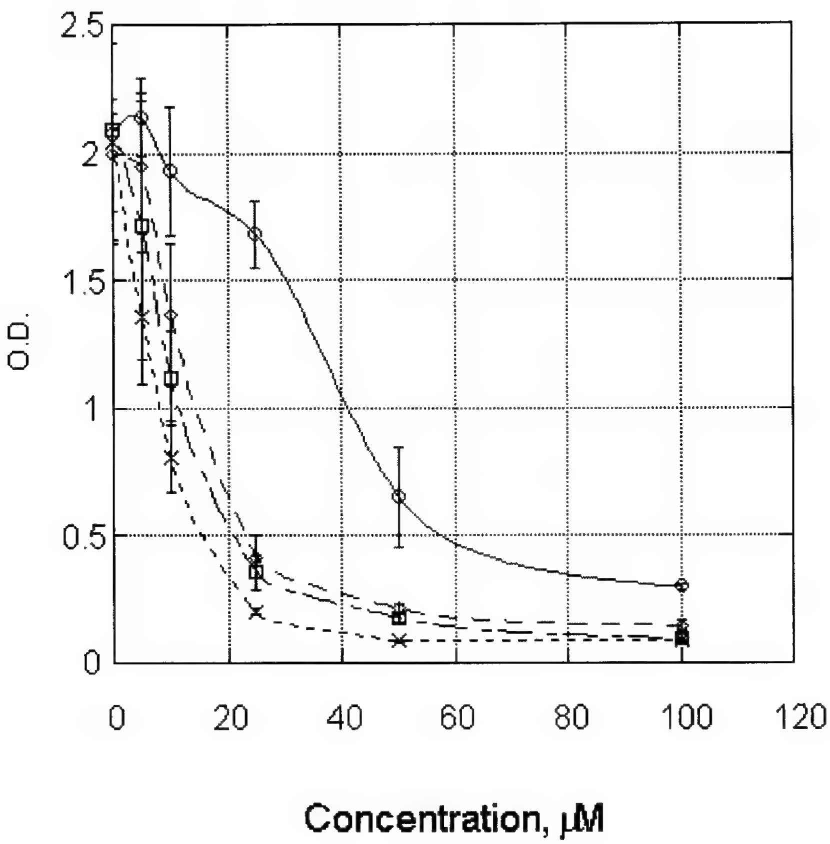


Figure 7. Effect of Pt complexes on the growth of the PA-1 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-09 (circle), Pt-10 (rectangle), Pt-11 (diamond), and carboplatin (cross).

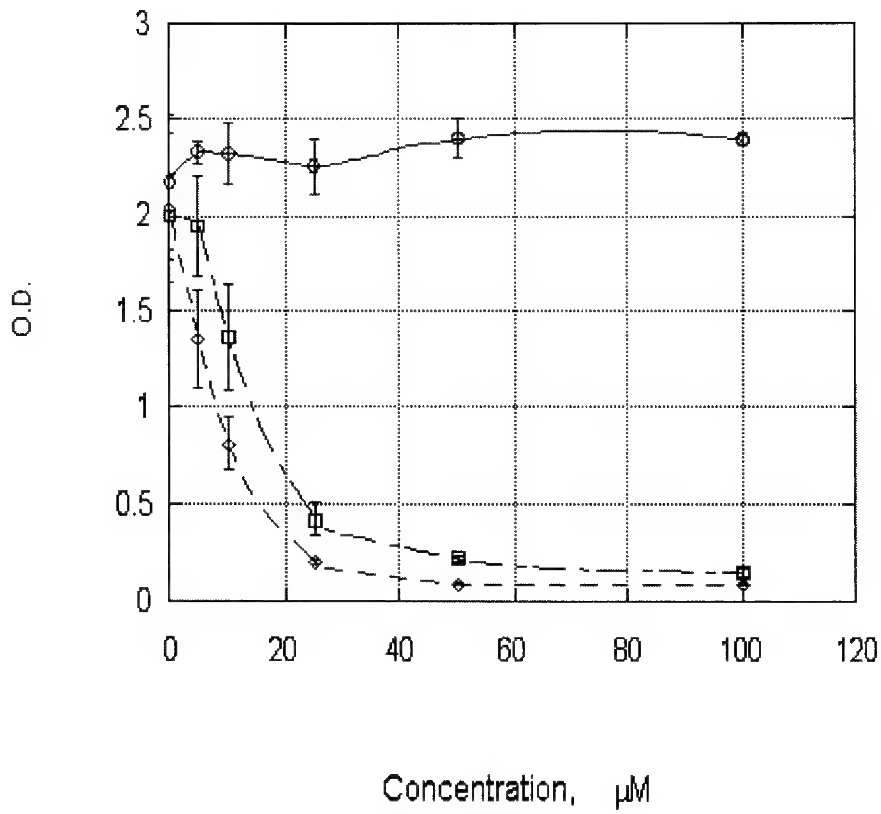


Figure 8. Effect of Pt-11 (complex) and K2-11 (salt) on the growth of the PA-1 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. K2-11 (circle), Pt-11 (rectangle), and carboplatin (diamond).

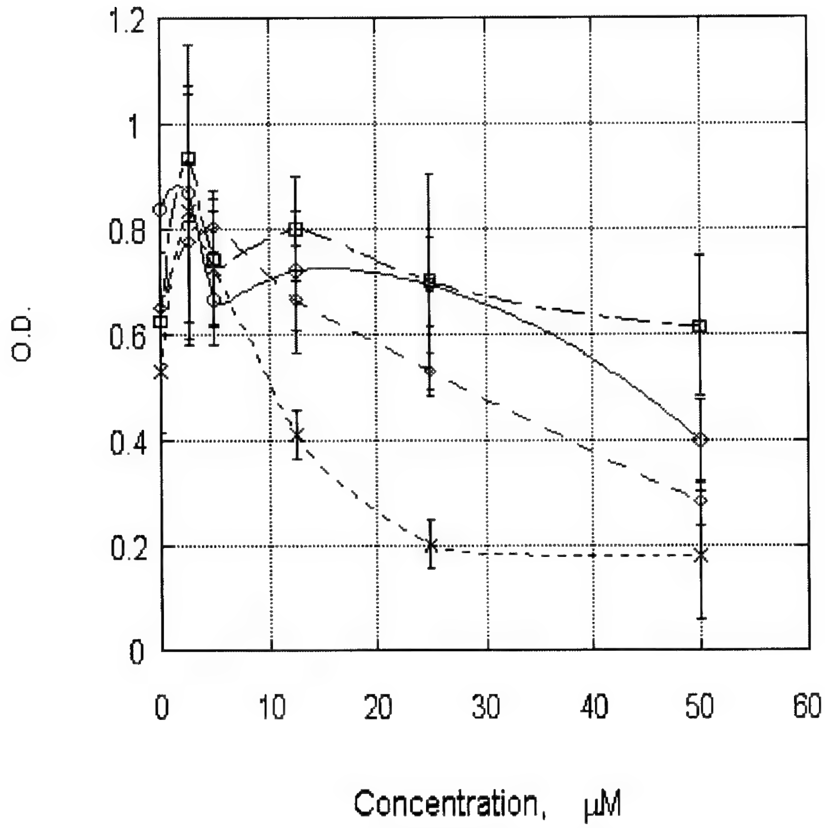


Figure 9. Effect of Pt conjugates on the growth of the SK-OV-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. Pt-09 (circle), Pt-10 (rectangle), Pt-11 (diamond), and carboplatin (cross).

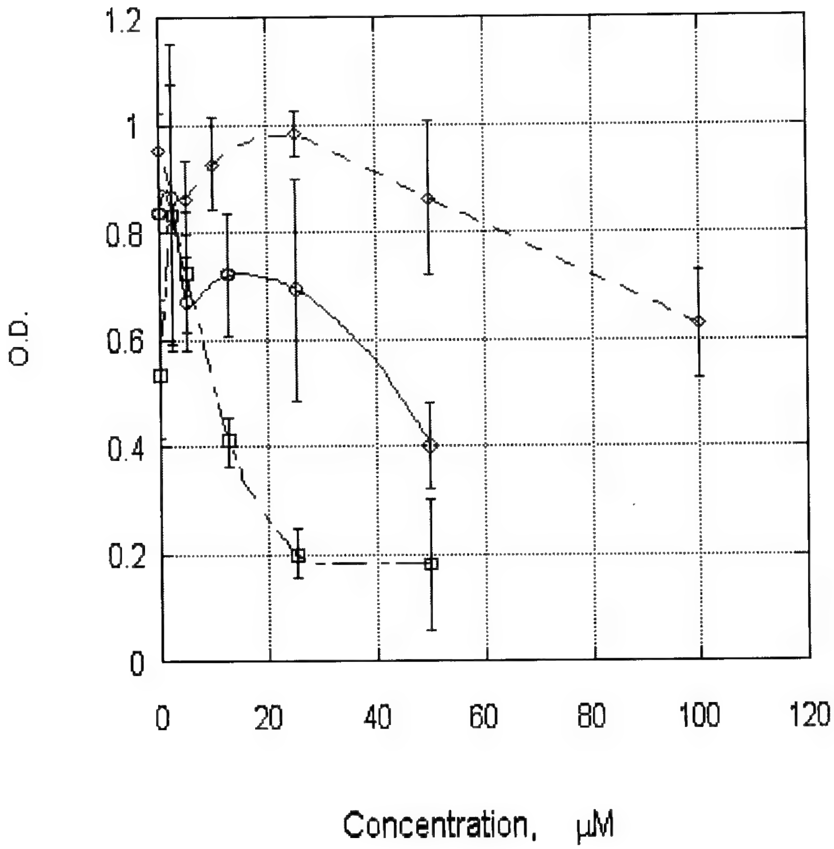


Figure 10. Effect of Pt-09 (complex) and K2-09 (salt) on the growth of the SK-OV-3 ovarian cancer cell line. IC_{50} values represent the concentrations of the conjugates which reduce the cell survival by 50%. K2-09 (diamond), Pt-09 (circle),and carboplatin (rectangle).

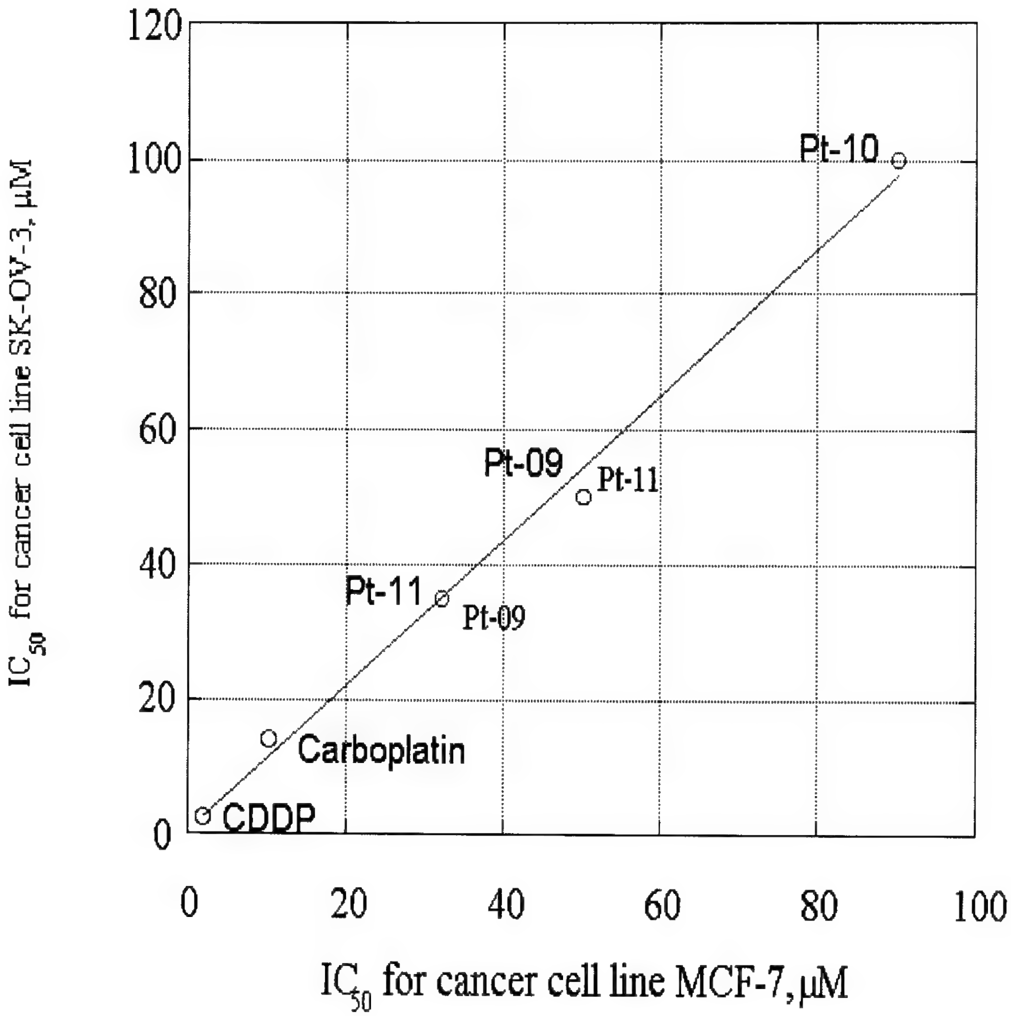


Figure 11. A Correlation diagram of IC_{50} between SK-OV-3 ovarian cancer cell line and MCF-7 breast cancer cell line. The IC_{50} values for SK-OV-3 was correlated to those for MCF-7 using a simple linear equation with the correlation coefficient of 0.997. The slope is calculated to be 1.08.

Synthesis and in Vitro Antitumor Activity of Oligonucleotide-Tethered and Related Platinum Complexes

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Three classes of hydroxy-tethered platinum(II) complexes have been synthesized from K_2PtCl_4 and appropriate amino alcohols. A sequence of selective oxidation and hydrolysis has been developed to prepare hydroxy-tethered platinum(IV) complexes. A novel procedure for the synthesis of amminetrichloroplatinate(II) anion has been generated and used to synthesize a number of monohydroxy-tethered nonchelating platinum complexes. These tethered platinum complexes, including hydroxy-tethered, phosphoramidite-tethered, and monodeoxyribonucleotide-tethered platinum(II) and -(IV) complexes, have been examined in vitro for antitumor activity in both leukemia and ovarian cancer cell lines. Activity of some of these complexes was similar to *cis*-platin, and most of them showed much better potency than carboplatin. We observed an interesting structure–activity correlation for platinum(II) complexes for both PA-1 and SK-OV-3 ovarian cancer cell lines. However, platinum(IV) complexes showed much more diversified response among cancer cell lines studied. We observed enhanced selectivity among different cancer cell lines for some agents. The most promising is the monodeoxyribonucleotide-tethered platinum(IV) complex, which is the first analogue of the conjugates between a platinum fragment and monodeoxyribonucleotides, showing antitumor activity and selectivity among the cell lines. Finally, the p53 status of the cells appears to contribute to the effectiveness of these agents in that cells harboring wild-type p53 appear to be more sensitive to these agents.

Introduction

A current approach to the design of novel platinum (Pt) drugs is to target the Pt coordination moiety to DNA by attaching it to a suitable carrier ligand. A variety of ligands have been attached to Pt, including DNA intercalators, doxorubicin, estrogen analogues, amino acids, sugars, and antitrypanosomal drugs.[1] Studies of Pt compounds with biologically active carrier groups have yielded interesting results, such as improved activity in cisplatin resistant cell lines[2] and interesting structure–activity relationships (SAR).[3] Despite these different structure–activity relationships, still lacking are platinum compounds suitable as synthons in the synthesis of the conjugates. Although conjugates between platinum and oligodeoxynucleotides have been reported before,[4] there have been no prior reports of a physiologically active platinum center in the conjugates as we will report in the present study. Here we synthesized a variety of tethered platinum complexes and examined them for biological activity in vitro. These include hydroxy-tethered, phosphoramidite-tethered, and monodeoxyribonucleotide-tethered platinum complexes, and we examined their actions in leukemia and ovarian cancer cell lines.

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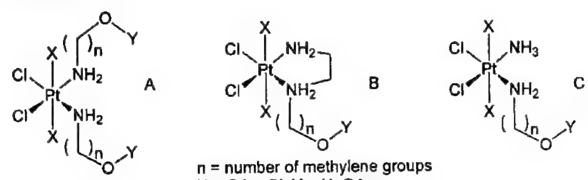
Scheme 1. Three General Classes of Platinum Compounds



Materials and Methods

The sketch of the compounds reported in this study is illustrated in Scheme 1. We will refer to these new agents as complexes A–C, each of which varies in the amine ligand.

Chemicals and Supplies for Synthesis. Potassium tetrachloroplatinate(II) (K_2PtCl_4) and potassium hexachloroplatinate(IV) (K_2PtCl_6) were purchased from Sausville Chemical Company (Garfield, NJ); phosphoric acid (H_3PO_4, 85%), hydrogen peroxide (30%), acetic acid HOAc), dimethylformamide (DMF), acetonitrile, methylene chloride, and ammonium chloride from Fisher Scientific (Pittsburgh, PA); acetic anhydride from Mallinckrodt Chemical (Paris, KY); hydrochloric acid (HCl) from EM Science (Gibbstown, NJ); deuterated dimethyl sulfoxide, water, methanol, and acetonitrile from Cambridge Isotope Company (Cambridge, MA); *N,N*-dimethylacetamide, 2-hydroxyethylamine, 3-hydroxypropylamine, 4-hydroxybutylamine, and tetraethylammonium chloride from Aldrich (Milwaukee, WI); and crystal violet and MTT from Sigma (St. Louis, MO). Compounds **8–10** and **20–22** were synthesized according to published procedures.[5] Elemental analyses were performed by Midwest Microlab (Indianapolis, IN).

NMR Measurements. All platinum(II) complexes undergo solvolysis at various rates in DMSO solutions. A minimum time exposure is required when conducting NMR studies in this solvent. Because of solubility limitations of some platinum(II) complexes, use of this solvent is sometimes unavoidable.

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The ^1H and $^{13}C\{^1H\}$ NMR spectra were obtained on either a Bruker Advance DRX/DPX 400 or 500 NMR spectrometer, and the chemical shifts were referenced to the residual solvent peaks. Standard parameters were used. The ^{195}Pt NMR spectra were obtained on a Varian 300 spectrometer, and the chemical shifts were referenced to *cis*-DDP in DMSO-d_6 at δ −2124 ppm, which was calibrated by using K_2PtCl_4 in water (δ −1623 ppm relative to Na_2PtCl_6) as an external standard. Typical parameters were the following: frequency at 64.324 MHz for platinum(II) and 64.494 MHz for platinum(IV) complexes, spectral width of 100 000 Hz, acqusition time of 6 ms, relaxation delay of 0 s, pulse width of 14 μs, temperature of 23 °C, decoupler off, line broadening of 150 Hz, and FT size of 1024.

Syntheses. Dichlorodi(3-hydroxypropylamine)platinum(II), 2. K_2PtCl_4 (0.500 g, 1.20 mmol) was dissolved in 4 mL of H_2O. 3-Hydroxypropylamine (221 mg, 2.2 eq) was added in one portion. The solution was stirred at room temperature (RT) for 4 h in the dark. The solvent was removed, and the solid was washed with acetic acid to get a yellow oily solid. The solid was extracted by CH_3OH to get rid of KCl. The CH_3OH solution was passed through a column of Dowex-X8 H^+ form 20−50 mesh cation-exchange resin by using CH_3OH as eluate. Removal of the solvent yielded a yellow powder of 261 mg; yield 52%. ^1H NMR in CD_3OD: δ 4.780 (br s, 4H, NH_2), 3.721 (t, $^3J_{HH}$ = 6.0 Hz, 2H, CH_2O), 2.931 (pent, $^3J_{HH}$ = 6.7 Hz, 2H, CH_2N), 1.958 (pent, $^3J_{HH}$ = 6.5 Hz, 2H, CH_2-CH_2O). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 61.2 (CH_2-O), 46.6 (CH_2-N), 34.3 (CH_2-CH_2O). ^{195}Pt NMR in CD_3OD: δ −2255. Elemental analysis, found: C, 17.31; H, 4.27; N, 6.60. Calcd for $C_6H_{18}N_2OCl_2Pt$: C, 17.31; H, 4.36; N, 6.73.

Dichlorodi(4-hydroxybutylamine)platinum(II), 3. K_2PtCl_4 (0.500 g, 1.20 mmol) was dissolved in 4 mL of H_2O. 4-Hydroxybutylamine (236 mg, 2.2 equiv) was added in one portion. The solution was stirred at RT for 4 h in the dark. The solvent was removed, and the solid was extracted by CH_3OH to get rid of KCl, yielding a yellow waxy solid, which was washed with acetic acid and diethyl ether to obtain a yellow powder of 310 mg; yield 58%. ^1H NMR in CD_3OD: δ 4.780 (brs, 4H, NH_2), 3.606 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2O), 2.806 (pent, $^3J_{HH}$ = 6.5 Hz, 2H, CH_2N), 1.780 (pent, $^3J_{HH}$ = 7.0 Hz, 2H, CH_2-CH_2O), 1.609 (pent, $^3J_{HH}$ = 6.6 Hz, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 62.6 (CH_2-O), 48.4 (CH_2-N), 30.8 (CH_2-CH_2O), 28.9 (CH_2-CH_2N). ^{195}Pt in CD_3OD, δ: −2224. Elemental analysis, found: C, 21.69; H, 4.85; N, 5.98. Calcd for $C_8H_{22}N_2OCl_2Pt$: C, 21.63; H, 4.99; N, 6.30.

Tetrachlorodi(3-hydroxypropylamine)platinum(IV), 6. Complex **2** (260 mg, 0.625 mmol) was suspended in 3.0 mL of acetic acid. Acetic anhydride (500 mg, 4.90 mmol) and 30% H_2O_2 (100 mg, 1.5 equiv) were added sequentially. After being stirred for about 30 min at RT, the mixture changed to a clear-yellow solution. CH_3OH (0.5 mL) was added to quench the reaction. Upon removal of the solvent, the solid was dissolved in 3 mL of 15% HCl solution. After the mixture was stirred at RT for 6 h, the solvent was removed, and the solid was washed with diethyl ether to get a yellow powder of 185 mg; yield 61%. ^1H NMR in DMSO: δ 6.423 (br s, 4H, NH_2), 3.394 (m, 2H, CH_2O), 2.874 (m, 2H, CH_2N), 1.838 (m, 2H, CH_2-CH_2O). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.3 (CH_2-O), 45.6 (CH_2-N), 32.4 (CH_2-CH_2O). ^{195}Pt NMR in DMSO: δ −146. Elemental analysis, found: C, 15.77; H, 3.80; N, 5.45; Pt, 39.2. Calcd for $C_6H_{18}N_2OCl_4Pt$: C, 14.79; H, 3.72; N, 5.75; Pt, 40.05.

Tetrachlorodi(4-hydroxybutylamine)platinum(IV), 7. The procedure was the same as that used for **6**; yield 69%. ^1H NMR in DMSO-d_6: δ 6.490 (brs, 4H, NH_2), 3.380 (m, 2H, CH_2O), 2.740 (m, 2H, CH_2N), 1.711 (m, 2H, CH_2-CH_2O), 1.419 (m, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 61.2 (CH_2-O), 47.1 (CH_2-N), 30.9 (CH_2-CH_2O), 26.5 (CH_2-CH_2N). ^{195}Pt in DMSO δ: −152. Elemental analysis, found: C, 18.41; H, 4.28; N, 5.26. Calcd for $C_8H_{22}N_2OCl_4Pt$: C, 18.65; H, 4.30; N, 5.44.

Potassium Amminetrichloroplatinate(II) [KPtCl$_3$(NH$_3$)], 11. *Cis*-DDP (4.0 g, 13.3 mmol), tetraethylammonium chloride (Et_4NCl, 2.82 g, 16.0 mmol), and ammonium chloride (NH_4Cl, 0.20 g, 3.7 mmol) were dissolved in *N,N*-dimethylacetamide (150 mL). The reaction mixture was heated at 100 °C, purging with a slow stream of N_2 for 8−10 h. The color of the solution changed from yellow to orange during this period. No black precipitate was observed. After the reaction, the solvent was partially removed down to 50 mL. Hexane/ethyl acetate (1:1, 300 mL) was then added, and the mixture was kept overnight at −20 °C. The supernatant was then decanted, and the solid was extracted with 60 mL of CH_3CN. The remaining solid (probably the mixture of NH_4Cl and *cis*-DDP) was washed with water to recover unreacted *cis*-DDP, 1.3 g. Water (5 mL) was added to the CH_3CN solution, and CH_3CN was removed by a mechanical pump, as judged by the volume of the solution. Dowex 50 W-X8 H^+ exchange resin (50 mL) was added, and the mixture was kept stirring for 1 h at RT. The resin was removed by filtration, and the solution was concentrated to 2 mL. Saturated KCl solution (5 mL) was added with stirring, and the mixture was kept at 0 °C for 2 h. The precipitate was collected and dried by air to get 2.8 g of $K[PtCl_3(NH_3)]\cdot(^1/_2)H_2O$. The yield was 58% based on *cis*-DDP added and 87% based on the *cis*-DDP consumed. The recovered *cis*-DDP is reusable. ^{195}Pt NMR in H_2O: δ −1888. Elemental analysis, found: Cl, 28.90; H, 1.55; N, 4.82. Calcd for $K[PtCl_3(NH_3)]\cdot(^1/_2)H_2O$: Cl, 29.01; H, 1.10; N, 3.82.

Amminechloroiodo(2-hydroxyethylamine)platinum(II), 12. Complex **11** (300 mg, 0.818 mmol) was dissolved in 2.0 mL of H_2O. NaI (240 mg, 1.9 eq) and 2-hydroxyethylamine (1.1 equiv) were added sequentially. The color of the solution changed from orange to red. Yellow precipitate began to form after about 5 min. The mixture was kept at 0 °C for 1 h. The solid was filtered and washed with precooled water and a small amount of diethyl ether to obtain 210 mg of **13**; yield 59%. ^1H NMR in CD_3OD: δ 3.888 (t, $^3J_{HH}$ = 5.0 Hz, 2H, CH_2O), 2.882 (pent, $^3J_{HH}$ = 5.3 Hz, 2H, CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.7 (CH_2-O), 47.9 (CH_2-N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ 62.3 (CH_2-O), 50.4 (CH_2-N). ^{195}Pt NMR in DMF: δ −2685. Elemental analysis, found: C, 5.54; H, 2.26; N, 6.21. Calcd for $C_2H_{10}N_2OClIPt$: C, 5.52; H, 2.31; N, 6.43.

Amminechloroiodo(4-hydroxybutylamine)platinum(II), 13. Complex **11** (300 mg, 0.818 mmol) was dissolved in 1.5 mL of H_2O. NaI (240 mg, 1.9 eq) was added, and the color of the solution changed from orange to red. 4-Hydroxybutylamine (1.1 equiv) was added. Yellow precipitate began to form immediately. The mixture was kept at 0 °C for 1 h. The solid was filtered and washed with precooled water and a small amount of acetone (0.5 mL) to obtain 270 mg of **13**; yield 71%. ^1H NMR in CD_3OD: δ 4.629 (brs, 3H, NH_3), 3.607 (t, $^3J_{HH}$ = 6.4 Hz, 2H, CH_2O), 2.810 (pent, $^3J_{HH}$ = 7.5 Hz, 2H, CH_2N), 1.802 (pent, $^3J_{HH}$ = 7.6 Hz, 2H, CH_2-CH_2O), 1.608 (pent, $^3J_{HH}$ = 7.5 Hz, 2H, CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 61.2 (CH_2-O), 46.0 (CH_2-N), 30.2 (CH_2-CH_2O), 27.8 (CH_2-CH_2N). $^{13}C\{^1H\}$ NMR in CD_3OD: δ: 62.4 (CH_2-O), 46.9 (CH_2-N), 30.7 (CH_2-CH_2O), 28.9 (CH_2-CH_2N). ^{195}Pt NMR in CD_3OD: δ −2718. Elemental analysis, found: C, 10.12; H, 2.95; N, 5.85. Calcd for $C_4H_{14}N_2OClIPt$: C, 10.36; H, 3.04; N, 6.04.

Amminedichloro(2-hydroxyethylamine)platinum(II), 14. Complex **12** (160 mg, 0.367 mmol) was suspended in 2.0 mL of H_2O. $AgNO_3$ (1.6 equiv) was added, and a light-yellow precipitate formed immediately. The mixture was stirred at RT for 4 h in the dark or until the solution was found to be Ag^+-free. Activated carbon (0.1 g) was added, and the mixture was stirred for 30 min. Concentrated HCl (1.0 mL) was added, and the solution was stirred for 30 min. The volatile was removed, and the solid was washed with diethyl ether and dried to afford 81 mg of **14**; yield 64%. ^1H NMR in DMSO-d_6: δ 4.712 (brs, 3H, NH_3), 3.983 (brs, 2H, NH_2), 3.574 (t, $^3J_{HH}$ = 5.5 Hz, 2H, CH_2OH), 2.609 (pent, $^3J_{HH}$ = 6.1 Hz, 2H, CH_2N). ^1H NMR in CD_3OD: δ 3.812 (t, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2O), 2.766 (pent, $^3J_{HH}$ = 5.5 Hz, 2H, CH_2N). $^{13}C\{^1H\}$ NMR in DMSO-d_6: δ 60.8 (CH_2-O), 49.2 (CH_2-N). ^{195}Pt NMR in DMSO: δ −2183. Elemental analysis, found: C, 7.11; H, 2.98; N, 7.90; Pt, 56.4. Calcd for $C_2H_{10}N_2OCl_2Pt$: C, 6.98; H, 2.93; N, 7.90; Pt, 56.4.

Amminedichloro(4-hydroxybutylamine)platinum(II), 15. The synthetic procedure is the same as that for **14**.

Briefly, complex **13** (160 mg, 0.345 mmol) was suspended in 2.0 mL of H_2O. $AgNO_3$ (1.6 equiv) was added, and the mixture was stirred at RT for 4 h in the dark or until the solution was found to be Ag^+-free. Activated carbon (0.1 g) was added, and the mixture was stirred for 0.5 h. Concentrated HCl (1.0 mL) was added, and the solution was stirred for 0.5 h. The volatile was removed, and the solid was washed with diethyl ether and dried to afford 101 mg of **15**. Yield 79%. ^{1}H NMR in DMSO-d_6: δ 4.711 (t, $^3J_{HH}$ = 5.0 Hz, 3H, NH_3), 3.975 (brs, 2H, NH_2), 3.385 (q, $^3J_{HH}$ = 5.3 Hz, 2H, CH_2OH), 2.513 (m, 2H, CH_2N), 1.566 (pent, $^3J_{HH}$ = 7.7 Hz, 2H, $CH_2{-}CH_2O$), 1.423 (pent, $^3J_{HH}$ = 7.1 Hz, 2H, $CH_2{-}CH_2N$). ^{1}H NMR in CD_3OD/acetone-d_6 (1:1): δ 4.612 (brs, 3H, NH_3), 3.503 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2O), 2.747 (pent, $^3J_{HH}$ = 6.9 Hz, 2H, CH_2N), 1.738 (pent, $^3J_{HH}$ = 7.4 Hz, 2H, $CH_2{-}CH_2O$), 1.515 (pent, $^3J_{HH}$ = 7.4 Hz, 2H, $CH_2{-}CH_2N$). ^{13}C{^{1}H} NMR in DMSO-d_6: δ 61.3 ($CH_2{-}O$), 47.3 ($CH_2{-}N$), 30.2 ($CH_2{-}CH_2O$), 27.7 ($CH_2{-}CH_2N$). ^{195}Pt NMR in H_2O: δ −2207. ^{195}Pt NMR in CD_3OD: δ −2189. Elemental analysis, found: C, 12.75; H, 3.72; N, 7.30. Calcd for $C_4H_{14}N_2OCl_2Pt$: C, 12.91; H, 3.79; N, 7.53.

Amminetetrachloro(2-hydroxyethylamine)platinum(IV), 18. Compound **14** (85 mg, 0.247 mmol) was suspended in 1 mL of HOAc. Acetic anhydride (500 mg, 20 equiv) and H_2O_2 30% solution (121 mg, 4.5 equiv) were added sequentially. Stirring the mixture for 2 h at RT solubilized it. Methanol was added to quench the reaction, and the solvent was removed. The solid was dissolved in 3 mL of 15% HCl solution, and the solution was stirred at RT for 6 h. The solvent was removed, and the solid was washed with diethyl ether and dried, yielding 88 mg of the yellow product **18**; yield 86%. ^{1}H NMR in D_2O: δ 3.763 (t, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2OH), 3.043 (pent, $^3J_{HH}$ = 5.2 Hz, 2H, CH_2N). ^{13}C{^{1}H} NMR in D_2O: δ 59.0 ($CH_2{-}O$), 47.7 ($CH_2{-}N$). ^{195}Pt NMR in D_2O: δ −334. Elemental analysis, found: C, 5.84; H, 2.43; N, 6.55; Pt, 47.00. Calcd for $C_2H_{10}N_2OCl_2Pt$: C, 5.79; H, 2.43; N, 6.75; Pt, 47.00.

Amminetetrachloro(4-hydroxybutylamine)platinum(IV), 19. Compound **15** (170 mg, 0.457 mmol) was suspended in 5 mL of HOAc. Acetic anhydride (400 mg, 8.6 equiv) and H_2O_2 30% solution (80 mg, 4.5 equiv) were added sequentially. The mixture was stirred for 0.5 h at RT, solubilizing it. Methanol was added to quench the reaction, and the solvent was removed. The solid was dissolved in 3 mL of 15% HCl solution, and the solution was stirred at RT for 6 h. The solvent was removed, and the solid was washed with diethyl ether and dried to yield 141 mg of the yellow product **19**; yield 70%. ^{1}H NMR in D_2O: δ 3.617 (t, $^3J_{HH}$ = 6.3 Hz, 2H, CH_2OH), 2.929 (t, $^3J_{HH}$ = 7.4 Hz, 2H, CH_2N), 1.767 (pent, $^3J_{HH}$ = 7.6 Hz, 2H, $CH_2{-}CH_2O$), 1.616 (pent, $^3J_{HH}$ = 7.2 Hz, 2H, $CH_2{-}CH_2N$). ^{13}C{^{1}H} NMR in D_2O: δ 60.9 ($CH_2{-}O$), 46.2 ($CH_2{-}N$), 28.6 ($CH_2{-}CH_2O$), 25.7 ($CH_2{-}CH_2N$). ^{195}Pt NMR in D_2O: δ −316. Elemental analysis, found: C, 10.80; H, 3.20; N, 6.18; Pt, 43.5. Calcd for $C_4H_{14}N_2OCl_4Pt$: C, 10.84; H, 3.18; N, 6.32; Pt, 44.03.

Cell Culture and Cytotoxicity for Leukemia Cancer Cell Lines. Teniposide (VM-26)-resistant leukemia cells, CEM/VM-1,[6] and the parental cell line, CEM, were cultured in minimum essential medium (BioWhittaker, Walkersville, MD) for suspension cells with 10% fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO) and 2 mM L-glutamine (BioWhittaker). The cells were incubated at 37 °C with 5% CO_2. Exponentially growing CEM and CEM/VM-1 cells were seeded into 24-well plates in triplicate at a density of 3×10^5 cells/mL. All drug solutions were prepared in cell culture medium by first dissolving in DMSO or PBS, then using serial dilutions to twice the desired concentration, and adding to the plates at equal volume. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 48 h. Cell growth inhibition was determined by counting on a Coulter Multisizer (Coulter Corporation, Miami, FL). The drug concentrations were plotted versus the percent of cell growth inhibition. The drug concentrations that inhibited cell growth by 50% were calculated by extrapolation and taken as the IC_{50} values.[7]

Colony Formation and Crystal Violet Assay for Ovarian Cancer Cell Lines. The ovarian cancer cell lines, PA-1 (wild-type [wt] p53), A2780 (wtp53), SW626 (mutant [m] p53), and SKOV-3 (p53-null) were obtained from American Type Culture Collection (ATCC) (Rockville, MD). These monolayer cells were cultured in RPMI 1640 (BioWhittaker) with 10% FBS (Sigma Chemical Co.) and 2 mM L-glutamine (BioWhittaker) and incubated at 37 °C in an atmosphere of 5% CO_2. Cell culture medium was aspirated from exponentially growing ovarian cells. The cells were washed with 1 × PBS, removed from the plate using a Trypsin−Versene mixture (BioWhittaker) and seeded in 6-well plates at a density of 300−500 cells/well. The cells were incubated 1 day in order to allow their proper attachment to the plates. Drug solutions were prepared in cell culture medium using serial dilutions and added to the cells in various concentrations as described above. All cells were incubated at 37 °C in an atmosphere of 5% CO_2. However, because of the varying growth rates of the ovarian lines, the PA-1 and A2780 cells were incubated with drug for 7 days and the SW626 and SKOV-3 cells were incubated with drug for 10 days. Following incubation, the cell culture medium was aspirated, the cells were washed with 1X PBS and fixed and stained using 0.1% crystal violet (Sigma) in methanol, and the colonies were counted using a colony counter (Artek Systems Corporation, Farmingdale, NY). The drug concentrations were plotted versus the percent inhibition of colony formation. Drug concentrations that inhibited colony formation by 50% were calculated by extrapolation and taken as IC_{50} values.

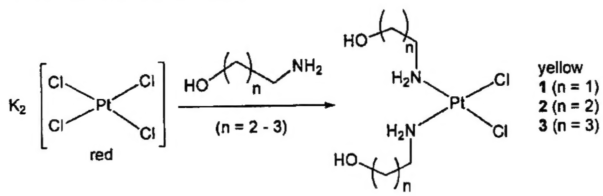
MTT Assay for Ovarian Cancer Cell Lines. The ovarian cancer cell lines, PA-1 (wild-type [wt] p53) and SKOV-3 (p53-null) were used for cytotoxic evaluation of some compounds. These monolayer cells were cultured in RPMI-1640 (BioWhittaker) with 10% FBS (Sigma Chemical Co.) and 2 mM L-glutamine (BioWhittaker) and incubated at 37 °C in an atmosphere of 5% CO_2. Cell culture medium was aspirated from exponentially growing ovarian cells. The cells were removed from the plate using a Trypsin−Versene mixture (BioWhittaker) and seeded in 96-well plates at a density of 1000 cells/well for PA-1 and 600 cells/well for SK-OV-3. The PA-1 cells were incubated for 1 day and SK-OV-3 cells were incubated for 2 days to allow for their proper attachment to the plates. Drug solutions were prepared in cell culture medium using serial dilutions and added to the cells in various concentrations as described above. All cells were incubated at 37 °C in an atmosphere of 5% CO_2. However, because of different growth rates of the ovarian cancer cell lines, the PA-1 cells were incubated with drugs for 5 days and the SKOV-3 cells were incubated with drugs for 7 days. At the end of the incubation, MTT (from Sigma, 25 μL, 4 mg/mL) in media without FBS and L-Gln were added to each well. The plate was incubated for 5 h at 37 °C and centrifuged in swinging bucket rotors at 1200 rpm for 15 min. After removal of the media, DMSO (200 μL) was added into each well. The mixture was then incubated for 30 min. The plates were read spectroscopically using a dual-wavelength filter (570 and 630 nm). The concentrations of complexes that decreased absorption by 50% were calculated by extrapolation and taken as the IC_{50} values.

Results and Discussion

Synthesis of Hydroxy-Tethered Platinum(II) and Platinum(IV) Complexes. Normally, the synthesis of the aminoplatinum(II) complexes starts with the interaction of K_2PtCl_4 with appropriate amines. This is indeed the procedure for the synthesis of *cis*-DDP.[8] We used this method successfully to prepare **2** and **3** (Scheme 2). However, the same procedure with ethanol amine gave **1** along with some other unidentified products in an oily form. Multiple efforts to purify this compound were unsuccessful probably because of the coordinative ability of the nearby hydroxy group to form a chelating five-membered ring.

Transformation of the above platinum(II) complexes into kinetically inert platinum(IV) carboxylate complexes was attempted following procedures developed

Scheme 2. Synthesis of Dihydroxy-Tethered Platinum(II) Complexes



for the synthesis of orally active platinum(IV) antitumor agents.[8] However, the tethered hydroxy groups were transformed into the ester groups, preventing direct coupling with other molecules such as oligodeoxynucleotides. Multiple attempts to selectively cleave the esters on the side chains were not successful except for hydrolysis in 20% HCl solution, which resulted in complexes **6** and **7** (Scheme 3). The same complexes were generated by the direct substitution of chlorides of K_2PtCl_6 by the corresponding amines but in very low yield.

Two types of monohydroxy-tethered platinum complexes have been developed. One involves a chelating diamino alcohol ligand.[5] Interaction of the ligand with K_2PtCl_4 generated platinum(II) complex **8**. Oxidation of **8** in the presence of acetic anhydride gave **9** with a free hydroxy group on the side chain. Hydrolysis in the presence of 20% HCl solution generated the corresponding tetrachloro amino platinum(IV) complex.[5b] The same compound, **10**, was also generated from the interaction of the chelating diamino alcohol with K_2PtCl_6 in low yield (Scheme 4).

However, the synthesis of monohydroxy-tethered platinum complexes without a chelating amino group was not straightforward (Scheme 5). Generation of the aminotrichloro platinum(II) anion, **11**, was first achieved by following a procedure in the literature.[5] However, our results by this method were low yield and lack of reproducibility. Multiple trials to improve the procedure resulted in our finding that a stabilizer, NH_4Cl, is critical for the consistent production of this starting material **11**. No decomposition was observed at prolonged heating (>18 h) at 110 °C or more than 1 h at 140 °C. It appears that the amount of NH_4Cl is not critical after a certain limit (from 0.25 to 2.0 molar equiv, no change in results was observed), probably maintaining a low concentration of NH_3 during the reaction. Without NH_4Cl as a stabilizer, a black precipitate began to form at less than 105 °C.

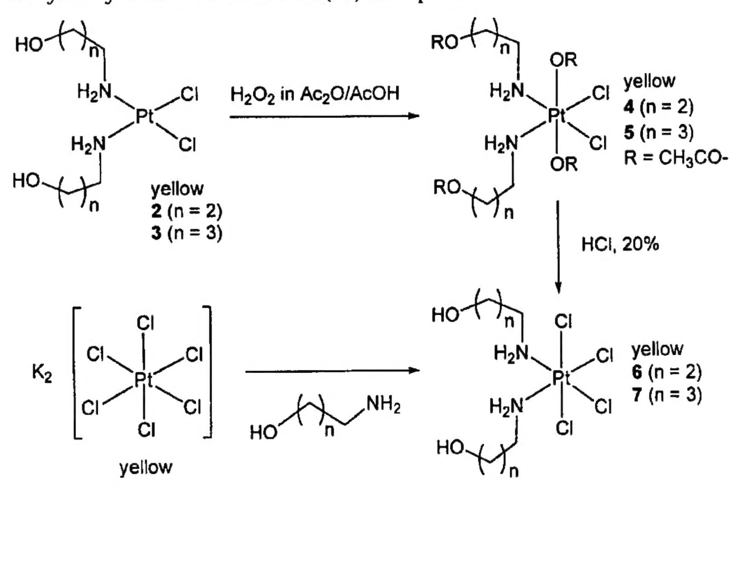
Replacement of the chloride by amines in the presence of NaI successfully gave **12** and **13**. Substitution of iodide with chloride in the presence of silver cations and subsequently quenched by HCl resulted in production of dichloro platinum(II) complexes **14** and **15**. At the last step, the reaction time is critical for its success. Prolonged reaction (overnight at RT, for example) tends to generate complicated mixtures. Monitoring of the reaction by ^{195}Pt NMR showed that the product formation is complete within 10 min. It appears that substitution of the iodide by chloride is critical for the subsequent oxidation; otherwise, a mixture was formed probably from partial iodo oxidation. H_2O_2 oxidation of **14** and **15** in the presence of acetic anhydride gave all protected complexes **16** and **17**. Subsequent hydrolysis in the presence of 20% HCl solution gave **18** and **19** with a free hydroxy group on the side chain ready for attachment of oligodeoxynucleotides.

Synthesis of Platinum Complexes Bearing Phosphoramidite Group and Monodeoxynucleotide-Tethered Platinum(II) and Platinum(IV) Complexes. These were synthesized according to methods previously reported from one of our laboratories (LC).[5] These are shown in Schemes 6 and 7.

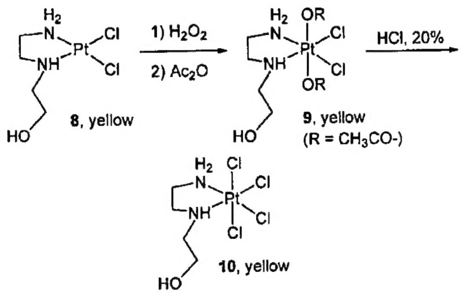
Cell Culture Studies of Tethered Platinum Complexes. The results of the growth inhibition and cytotoxicity studies of a series of our newly synthesized compounds are summarized in Tables 1 and 2, depending on the assay used to evaluate the compounds. The correlation of the IC_{50} values between those from PA-1 and SK-OV-3 is shown in Figure 1.

The dotted line represents the IC_{50} values from platinum(II) complexes and resembles an S-shaped curve. Except for *cis*-DDP, the curve follows an exponential function, with $IC_{50}(SKOV\text{-}3) = 17.5 + 28.4 \log[IC_{50}(PA\text{-}1)]$, $R = 0.999$. What is striking is the uniform change in potency for both cell lines, although a variety of differences exist for both cell lines, demonstrating that the major factors controlling the IC_{50} values were maintained for this group of platinum(II) complexes. The mechanism(s) that makes the SK-OV-3 more re-

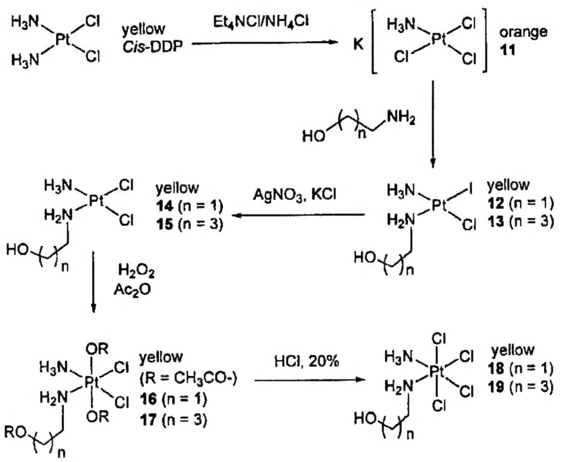
Scheme 3. Synthesis of Dihydroxy-Tethered Platinum(IV) Complexes



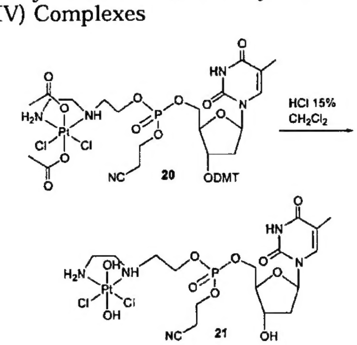
Scheme 4. Synthesis of Chelate Monohydroxy-Tethered Platinum(II) and Platinum(IV) Complexes



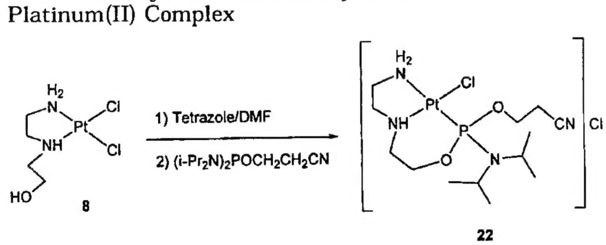
Scheme 5. Synthesis of Monohydroxy-Tethered Platinum(II) and Platinum(IV) Complexes



Scheme 6. Synthesis of Monodeoxynucleotide-Tethered Platinum(IV) Complexes



Scheme 7. Synthesis of a Phosphoramidite-Tethered Platinum(II) Complex



sistant toward platinum(II) complexes also appears to be the same for this group of compounds. Also of interest, the p53 status of the cells may be important for the action of these platinum(II) complexes. Thus, cells with wild-type p53 (PA-1 and A2780) are more sensitive to these complexes than those with mutant p53 (SW626 and SK-OV-3). Among the platinum(II) com-

Table 1. Cell Growth and Colony Formation Inhibition of Leukemia and Ovarian Cancer Cell Lines[a]

	cell line					
Pt complex	CEM[b]	CEM/ VM-1	PA-1	A2780	SW626	SKOV-3
cis-DDP	<1	<1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
6	8 ± 1	5 ± 0.8	0.6 ± 0.2	1.6 ± 0.3	3.0 ± 0.5	10.0 ± 1
7	10 ± 1	10 ± 1	1.0 ± 0.2	2.2 ± 0.3	7 ± 1	4 ± 0.5
9	7 ± 1	7 ± 1	1.5 ± 0.3	2.8 ± 0.2	28 ± 4	24 ± 3
20	35 ± 3	35 ± 3	3 ± 0.5	4 ± 0.5	30 ± 3	24 ± 2
21	100 ± 10	100 ± 10	13 ± 1	16 ± 2	40 ± 4	75 ± 8
22	none	none	165 ± 20	125 ± 20	>200	>200

[a] IC_{50} values of growth inhibition and colony formation were determined as described in Materials and Methods. Concentrations are in μM. Values are $X \pm$ SD of three separate experiments. [b] CEM and VM-1 are leukemia cancer cell lines (with MDR), and PA-1, A2780, SW626, and SKOV-3 are ovarian cancer cell lines.

Table 2. Cell Growth Inhibition of Ovarian Cancer Cell Lines[a]

	cell lines	
Pt compounds	PA-1	SK-OV-3
cis-DDP	0.08 ± 0.03	2.6 ± 0.5
15	0.4 ± 0.1	6.0 ± 1
14	0.7 ± 0.5	13 ± 3
2	1.2 ± 0.4	20 ± 4
3	1.5 ± 0.5	23 ± 5
carboplatin	2.4 ± 0.8	28.5 ± 6
8	2.9 ± 1.2	30 ± 6
19	0.2 ± 0.1	9 ± 2
17	0.8 ± 0.3	22 ± 5
18	0.9 ± 0.3	10 ± 2
10	1.7 ± 0.6	26 ± 5
16	2.8 ± 0.9	10 ± 2

[a] IC_{50} values of growth inhibition were determined by MTT assay as described in Materials and Methods. Concentrations are in μM. Values are $X \pm$ SD of four separate experiments.

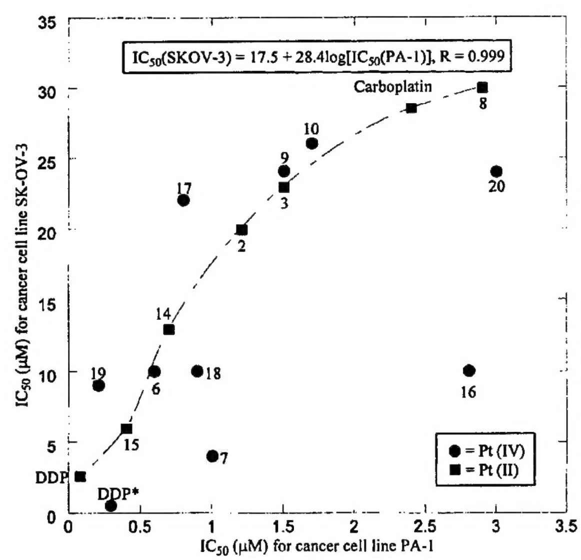


Figure 1. Correlation of IC_{50} values of the platinum complexes for cancer cell lines PA-1 and SK-OV-3. DDP represent the IC_{50} value from Table 1, and DDP* represents the IC_{50} value from Table 2. Squares represent platinum(II) complexes, and circles represent platinum(IV) complexes.

plexes examined, the following structural features appear to contribute to their potency. The chelated amine complex **8** had the lowest potency. The complexes with two amino alcohol ligands, **2** and **3**, performed better. It appears that some flexibility in the chain of the amino ligand is quite important. Monoamine complexes **14** and

15 performed better than diamine complexes **2** and **3**. This result suggests again that the flexibility of the ligand around the platinum(II) center plays a central role in the activity of the drugs. Another interesting result is that within each class of amino ligands, longer alkyl chains were more potent, and for two adjacent alkyl groups, those with odd numbers of methylene groups performed better.

Compared with platinum(II) complexes, the platinum(IV) complexes show much more varied responses in the two cell lines. No general correlation between IC_{50} values from the two cancer cell lines exists, as shown in Figure 1. In fact, platinum(IV) complexes **7**, **16**, **18**, and **20** show remarkable selectivity to the SK-OV-3 cancer cell line. The most striking examples are complexes **7** and **16**. If we accept that all platinum(IV) complexes are reduced to platinum(II) complexes before binding with DNA, more factors are involved for platinum(IV) complexes, such as the rate of reduction and transport. Recently, the direct interaction between platinum(IV) complexes and nucleotides has been demonstrated in solution chemistry.[9] These extra factors may render different selectivity for platinum(IV) complexes.

Although none of the new compounds are as potent as *cis*-DDP, several facts are evident. First, all platinum(IV) complexes examined are active cytotoxic agents. Second, compounds **6** and **7** (both are Pt(IV)) are the most active in all cell lines, with activities approaching that of *cis*-DDP. Third, the conjugates of platinum(IV) complexes and monodeoxynucleotides are active and show enhanced selectivity among different cancer cell lines. Fourth, and perhaps the most important, the compounds show activity comparable to that of *cis*-DDP in CEM/VM-1 cells that express multidrug resistance due in part to mutation in and decreased activity of topoisomerase II.[10]

This increased selectivity of the platinum(IV) complexes among these different cancer cell lines is an interesting result and suggests that the lower potency of our platinum complexes compared to *cis*-DDP may not be so important. The potency of an individual compound also appears to be affected by chemical groups far away from the platinum center, lending further support to the notion that our new agents are potential candidates as precursors of selective cytotoxic drugs. For example, for **9** vs **20**, the platinum center is the same. While **20** is less potent than **9** in PA-1, both are about the same for SK-OV-3.

The reduced potency of the mononucleotide–Pt conjugates relative to *cis*-DDP is not surprising. The mononucleotides alone show negligible affinity toward either mRNA or DNA. This likely comes from the observation that the minimum length of an ODN that is necessary for targeting a unique sequence in mRNA is approximately 15–20 bases.[11] The tether connecting the mononucleotide and Pt fragment consists of only two methylene groups. This may not be an optimized length for the simultaneous binding of a mononucleotide and Pt to the biological targets.

Our result does not support the general notion that Pt(IV) complexes are less potent than their Pt(II) counterparts;[12] instead, from the numerous examples we have shown here, the opposite is true, such as in **2** vs **6**, **3** vs **7**, **8** vs **9**, and **8** vs **10**. Only **15** vs **17** and **14** vs **18** show the normal pattern. Others such as **14** vs **16** and **14** vs **18** show different responses among different cancer cell lines.

The kinetically inert platinum(IV) complexes can serve as protecting elements to prevent the premature liberation of the platinum(II) center. Under physiological conditions, we expect that reduction of the platinum(IV) center by ascorbic acid or glutathione and a reductase enzyme should result in the liberation of the modified *cis*-DDP, and this should interact with mRNA or dsDNA. This type of reduction has been achieved with the orally administered drug *cis*-(ammine)(cyclohexylamine)dichloro-*trans*-(diacetato)platinum(IV)[13] and with our own in vitro study of the reaction of the platinum(IV) complexes with ascorbic acid or thiols.[14] With ascorbic acid (0.1 M), all platinum(IV) complexes were reduced to platinum(II) complexes within 1–2 days, and with thiols, they were reduced within 1–3 h, demonstrating the possibility of activation by the physiological reductants. Of importance in the present study, all of the platinum(IV) complexes we tested on cancer cell lines showed inhibition of cell growth or colony formation at low concentrations. This result suggests that platinum(IV) complexes can be activated under physiological conditions. However, we have not yet demonstrated this nor have we demonstrated that our compounds remain conjugated after they enter the cells.

Conclusions

We have prepared in the present study a series of hydroxy-tethered Pt(II) and Pt(IV) complexes that can serve as synthons to construct other conjugates of biological interest. These tethered platinum complexes and the conjugate between a platinum fragment with a monodeoxyribonucleotide have been evaluated in in vitro growth inhibition and cytotoxicity assays. Except for compound, **22**, which has a coordinated phosphoramidite group and was inactive, all other compounds were active and some revealed slightly less potency when compared to *cis*-DDP. For platinum(II) complexes, the response between PA-1 and SK-OV-3 is remarkably correlated, with PA-1 the more sensitive to the platinum complexes. Among the amine ligands examined, the potency of the platinum complexes increases from those of chelate diamine, two amines, to those of the monoamine ligands. All of the amine ligands benefits from long aliphatic chains, with slight favor for odd-numbered ones. Overall, the p53 status may be important in the actions of these agents. However, multidrug resistance associated with altered DNA topoisomerase II is not a factor in the actions of these compounds because they appear to have equivalent potency in these cells and their drug sensitive parents. For the platinum(IV) complexes, the response is more diversified. More factors are involved for the cytotoxicity for platinum(IV) complexes, including transport, reduction, and possibly direct interaction with DNA. Groups far away from a platinum center also have a substantial effect on the IC_{50} values. These tethered complexes demonstrate substantial selectivity toward the SK-OV-3 cancer cell line. In this regard, the p53 status may be a critical factor for the enhanced selectivity among different cancer cell lines, at least the ovarian carcinoma lines.

Thus, cells expressing the wild-type protein appear to be more sensitive to the changes of the tether than those with mutant p53. Finally, these conjugates between a platinum fragment and ODNs have the potential characteristics necessary for sequence-specific inhibition of oncogenes such as erbB-2, which is overexpressed in 25–30% of primary breast and ovarian cancer patients.[15] These compounds described in this study represent the first steps in the design of such sequence-specific inhibitions of oncogene expression.

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